

**RNA INTERFERENCE MEDIATED INHIBITION OF GPRA AND AAA1 GENE  
EXPRESSION USING SHORT INTERFERING NUCLEIC ACID (siNA)**

This application claims the benefit of U.S. Provisional Application No. 60/570,086, filed May 11, 2004. This application is a continuation-in-part of 5 International Patent Application No. PCT/US04/16390, filed May 24, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/826,966, filed April 16, 2004, which is continuation-in-part of U.S. Patent Application No. 10/757,803, filed January 14, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/720,448, filed November 24, 2003, which is a continuation-in-part of U.S. Patent Application No. 10/693,059, filed October 23, 2003, which is a continuation-in-part of U.S. Patent Application No. 10/444,853, filed May 23, 2003, which is a continuation-in-part of International Patent Application No. PCT/US03/05346, filed February 20, 2003, and a continuation-in-part of International Patent Application No. PCT/US03/05028, filed February 20, 2003, both of which claim the benefit of U.S. Provisional Application No. 15 60/358,580 filed February 20, 2002, U.S. Provisional Application No. 60/363,124 filed March 11, 2002, U.S. Provisional Application No. 60/386,782 filed June 6, 2002, U.S. Provisional Application No. 60/406,784 filed August 29, 2002, U.S. Provisional Application No. 60/408,378 filed September 5, 2002, U.S. Provisional Application No. 60/409,293 filed September 9, 2002, and U.S. Provisional Application No. 60/440,129 filed January 15, 2003. This application is also a continuation-in-part of International 20 Patent Application No. PCT/US04/13456, filed April 30, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/780,447, filed February 13, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/427,160, filed April 30, 2003, which is a continuation-in-part of International Patent Application No. PCT/US02/15876 25 filed May 17, 2002, which claims the benefit of U.S. Provisional Application No. 60/292,217, filed May 18, 2001, U.S. Provisional Application No. 60/362,016, filed March 6, 2002, U.S. Provisional Application No. 60/306,883, filed July 20, 2001, and U.S. Provisional Application No. 60/311,865, filed August 13, 2001. This application is also a continuation-in-part of U.S. Patent Application No. 10/727,780 filed December 3, 30 2003. This application also claims the benefit of U.S. Provisional Application No. 60/543,480, filed February 10, 2004. The instant application claims the benefit of all the

listed applications, which are hereby incorporated by reference herein in their entireties, including the drawings.

#### Field Of The Invention

The present invention relates to compounds, compositions, and methods for the 5 study, diagnosis, and treatment of traits, diseases and conditions that respond to the modulation of G protein-coupled receptor for asthma susceptibility (GPRA) and asthma-associated alternatively spliced gene 1 (AAA1) gene expression and/or activity. The present invention is also directed to compounds, compositions, and methods relating to traits, diseases and conditions that respond to the modulation of expression and/or 10 activity of genes involved in GPRA and/or AAA1 gene expression pathways or other cellular processes that mediate the maintenance or development of such traits, diseases and conditions. Specifically, the invention relates to small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules 15 capable of mediating RNA interference (RNAi) against GPRA and/or AAA1 gene expression. Such small nucleic acid molecules are useful, for example, in providing compositions for treatment of traits, diseases and conditions that can respond to modulation of GPRA and/or AAA1 expression in a subject, such as respiratory and/or inflammatory diseases, disorders, or conditions.

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#### Background Of The Invention

The following is a discussion of relevant art pertaining to RNAi. The discussion is provided only for understanding of the invention that follows. The summary is not an admission that any of the work described below is prior art to the claimed invention.

RNA interference refers to the process of sequence-specific post-transcriptional 25 gene silencing in animals mediated by short interfering RNAs (siRNAs) (Zamore *et al.*, 2000, *Cell*, 101, 25-33; Fire *et al.*, 1998, *Nature*, 391, 806; Hamilton *et al.*, 1999, *Science*, 286, 950-951; Lin *et al.*, 1999, *Nature*, 402, 128-129; Sharp, 1999, *Genes & Dev.*, 13:139-141; and Strauss, 1999, *Science*, 286, 886). The corresponding process in 30 plants (Heifetz *et al.*, International PCT Publication No. WO 99/61631) is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to

as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have 5 evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response through a mechanism that has yet to be fully characterized. This mechanism appears to 10 be different from other known mechanisms involving double stranded RNA-specific ribonucleases, such as the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L (see for example US Patent Nos. 6,107,094; 5,898,031; Clemens *et al.*, 1997, *J. Interferon & Cytokine Res.*, 17, 503-524; 15 Adah *et al.*, 2001, *Curr. Med. Chem.*, 8, 1189).

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer (Bass, 2000, *Cell*, 101, 235; Zamore *et al.*, 2000, *Cell*, 101, 25-33; Hammond *et al.*, 2000, *Nature*, 404, 293). Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) 20 (Zamore *et al.*, 2000, *Cell*, 101, 25-33; Bass, 2000, *Cell*, 101, 235; Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes (Zamore *et al.*, 2000, *Cell*, 101, 25-33; Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs 25 (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA 30 takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188).

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Bahramian and Zarbl, 1999, *Molecular and Cellular Biology*, 19, 274-283 and Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mammalian systems. Hammond *et al.*, 2000, 5 *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature*, 411, 494 and Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in *Drosophila* embryonic lysates (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877 10 and Tuschl *et al.*, International PCT Publication No. WO 01/75164) has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21-nucleotide siRNA duplexes are most active when containing 3'-terminal dinucleotide overhangs. Furthermore, complete substitution of one or both siRNA strands with 2'- 15 deoxy (2'-H) or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of the 3'-terminal siRNA overhang nucleotides with 2'-deoxy nucleotides (2'-H) was shown to be tolerated. Single mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide 20 sequence rather than the 3'-end of the guide sequence (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell*, 107, 309).

Studies have shown that replacing the 3'-terminal nucleotide overhanging segments 25 of a 21-mer siRNA duplex having two-nucleotide 3'-overhangs with deoxyribonucleotides does not have an adverse effect on RNAi activity. Replacing up to four nucleotides on each end of the siRNA with deoxyribonucleotides has been reported to be well tolerated, whereas complete substitution with deoxyribonucleotides results in no RNAi activity (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877 and Tuschl *et al.*, 30 International PCT Publication No. WO 01/75164). In addition, Elbashir *et al.*, *supra*, also report that substitution of siRNA with 2'-O-methyl nucleotides completely abolishes RNAi activity. Li *et al.*, International PCT Publication No. WO 00/44914, and Beach *et*

*al.*, International PCT Publication No. WO 01/68836 preliminarily suggest that siRNA may include modifications to either the phosphate-sugar backbone or the nucleoside to include at least one of a nitrogen or sulfur heteroatom, however, neither application postulates to what extent such modifications would be tolerated in siRNA molecules, nor 5 provides any further guidance or examples of such modified siRNA. Kreutzer *et al.*, Canadian Patent Application No. 2,359,180, also describe certain chemical modifications for use in dsRNA constructs in order to counteract activation of double-stranded RNA-dependent protein kinase PKR, specifically 2'-amino or 2'-O-methyl nucleotides, and nucleotides containing a 2'-O or 4'-C methylene bridge. However, Kreutzer *et al.* 10 similarly fails to provide examples or guidance as to what extent these modifications would be tolerated in dsRNA molecules.

Parrish *et al.*, 2000, *Molecular Cell*, 6, 1077-1087, tested certain chemical modifications targeting the unc-22 gene in *C. elegans* using long (>25 nt) siRNA transcripts. The authors describe the introduction of thiophosphate residues into these 15 siRNA transcripts by incorporating thiophosphate nucleotide analogs with T7 and T3 RNA polymerase and observed that RNAs with two phosphorothioate modified bases also had substantial decreases in effectiveness as RNAi. Further, Parrish *et al.* reported that phosphorothioate modification of more than two residues greatly destabilized the RNAs *in vitro* such that interference activities could not be assayed. *Id.* at 1081. The 20 authors also tested certain modifications at the 2'-position of the nucleotide sugar in the long siRNA transcripts and found that substituting deoxynucleotides for ribonucleotides produced a substantial decrease in interference activity, especially in the case of Uridine to Thymidine and/or Cytidine to deoxy-Cytidine substitutions. *Id.* In addition, the authors tested certain base modifications, including substituting, in sense and antisense 25 strands of the siRNA, 4-thiouracil, 5-bromouracil, 5-iodouracil, and 3-(aminoallyl)uracil for uracil, and inosine for guanosine. Whereas 4-thiouracil and 5-bromouracil substitution appeared to be tolerated, Parrish reported that inosine produced a substantial decrease in interference activity when incorporated in either strand. Parrish also reported that incorporation of 5-iodouracil and 3-(aminoallyl)uracil in the antisense strand 30 resulted in a substantial decrease in RNAi activity as well.

The use of longer dsRNA has been described. For example, Beach *et al.*, International PCT Publication No. WO 01/68836, describes specific methods for attenuating gene expression using endogenously-derived dsRNA. Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe a *Drosophila in vitro* RNAi system and the use of specific siRNA molecules for certain functional genomic and certain therapeutic applications; although Tuschl, 2001, *Chem. Biochem.*, 2, 239-245, doubts that RNAi can be used to cure genetic diseases or viral infection due to the danger of activating interferon response. Li *et al.*, International PCT Publication No. WO 00/44914, describe the use of specific long (141 bp-488 bp) enzymatically synthesized or 5 vector expressed dsRNAs for attenuating the expression of certain target genes. Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646, describe certain methods for inhibiting the expression of particular genes in mammalian cells using certain long (550 bp-714 bp), enzymatically synthesized or vector expressed dsRNA molecules. Fire *et al.*, International PCT Publication No. WO 99/32619, describe 10 particular methods for introducing certain long dsRNA molecules into cells for use in inhibiting gene expression in nematodes. Plaetinck *et al.*, International PCT Publication No. WO 00/01846, describe certain methods for identifying specific genes responsible for conferring a particular phenotype in a cell using specific long dsRNA molecules. Mello *et al.*, International PCT Publication No. WO 01/29058, describe the identification 15 of specific genes involved in dsRNA-mediated RNAi. Pachuck *et al.*, International PCT Publication No. WO 00/63364, describe certain long (at least 200 nucleotide) dsRNA constructs. Deschamps Depaillette *et al.*, International PCT Publication No. WO 99/07409, describe specific compositions consisting of particular dsRNA molecules combined with certain anti-viral agents. Waterhouse *et al.*, International PCT 20 Publication No. 99/53050 and 1998, *PNAS*, 95, 13959-13964, describe certain methods for decreasing the phenotypic expression of a nucleic acid in plant cells using certain dsRNAs. Driscoll *et al.*, International PCT Publication No. WO 01/49844, describe 25 specific DNA expression constructs for use in facilitating gene silencing in targeted organisms.

30 Others have reported on various RNAi and gene-silencing systems. For example, Parrish *et al.*, 2000, *Molecular Cell*, 6, 1077-1087, describe specific chemically-modified dsRNA constructs targeting the unc-22 gene of *C. elegans*. Grossniklaus, International

PCT Publication No. WO 01/38551, describes certain methods for regulating polycomb gene expression in plants using certain dsRNAs. Churikov *et al.*, International PCT Publication No. WO 01/42443, describe certain methods for modifying genetic characteristics of an organism using certain dsRNAs. Cogoni *et al.*, International PCT Publication No. WO 01/53475, describe certain methods for isolating a *Neurospora* silencing gene and uses thereof. Reed *et al.*, International PCT Publication No. WO 01/68836, describe certain methods for gene silencing in plants. Honer *et al.*, International PCT Publication No. WO 01/70944, describe certain methods of drug screening using transgenic nematodes as Parkinson's Disease models using certain dsRNAs. Deak *et al.*, International PCT Publication No. WO 01/72774, describe certain *Drosophila*-derived gene products that may be related to RNAi in *Drosophila*. Arndt *et al.*, International PCT Publication No. WO 01/92513 describe certain methods for mediating gene suppression by using factors that enhance RNAi. Tuschl *et al.*, International PCT Publication No. WO 02/44321, describe certain synthetic siRNA constructs. Pachuk *et al.*, International PCT Publication No. WO 00/63364, and Satishchandran *et al.*, International PCT Publication No. WO 01/04313, describe certain methods and compositions for inhibiting the function of certain polynucleotide sequences using certain long (over 250 bp), vector expressed dsRNAs. Echeverri *et al.*, International PCT Publication No. WO 02/38805, describe certain *C. elegans* genes identified via RNAi. Kreutzer *et al.*, International PCT Publications Nos. WO 02/055692, WO 02/055693, and EP 1144623 B1 describes certain methods for inhibiting gene expression using dsRNA. Graham *et al.*, International PCT Publications Nos. WO 99/49029 and WO 01/70949, and AU 4037501 describe certain vector expressed siRNA molecules. Fire *et al.*, US 6,506,559, describe certain methods for inhibiting gene expression in vitro using certain long dsRNA (299 bp-1033 bp) constructs that mediate RNAi. Martinez *et al.*, 2002, *Cell*, 110, 563-574, describe certain single stranded siRNA constructs, including certain 5'-phosphorylated single stranded siRNAs that mediate RNA interference in Hela cells. Harborth *et al.*, 2003, *Antisense & Nucleic Acid Drug Development*, 13, 83-105, describe certain chemically and structurally modified siRNA molecules. Chiu and Rana, 2003, *RNA*, 9, 1034-1048, describe certain chemically and structurally modified siRNA molecules. Woolf *et al.*, International PCT Publication

Nos. WO 03/064626 and WO 03/064625 describe certain chemically modified dsRNA constructs.

### SUMMARY OF THE INVENTION

This invention relates to compounds, compositions, and methods useful for modulating G protein-coupled receptor for asthma susceptibility (GPRA) and/or asthma-associated alternatively spliced gene 1 (AAA1) gene expression using short interfering nucleic acid (siNA) molecules. This invention also relates to compounds, compositions, and methods useful for modulating the expression and activity of other genes involved in pathways of GPRA and/or AAA1 gene expression and/or activity by RNA interference (RNAi) using small nucleic acid molecules. In particular, the instant invention features small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules and methods used to modulate the expression of GPRA and/or AAA1 genes.

A siNA of the invention can be unmodified or chemically-modified. A siNA of the instant invention can be chemically synthesized, expressed from a vector or enzymatically synthesized. The instant invention also features various chemically-modified synthetic short interfering nucleic acid (siNA) molecules capable of modulating GPRA and/or AAA1 gene expression or activity in cells by RNA interference (RNAi). The use of chemically-modified siNA improves various properties of native siNA molecules through increased resistance to nuclease degradation *in vivo* and/or through improved cellular uptake. Further, contrary to earlier published studies, siNA having multiple chemical modifications retains its RNAi activity. The siNA molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, veterinary, diagnostic, target validation, genomic discovery, genetic engineering, and pharmacogenomic applications.

In one embodiment, the invention features one or more siNA molecules and methods that independently or in combination modulate the expression of GPRA and/or AAA1 genes encoding proteins, such as proteins comprising GPRA and/or AAA1 associated with the maintenance and/or development of inflammatory and/or respiratory

diseases, traits, conditions and disorders, such as genes encoding sequences comprising those sequences referred to by GenBank Accession Nos. shown in **Table I**, referred to herein generally as GPRA and/or AAA1. The description below of the various aspects and embodiments of the invention is provided with reference to exemplary GPRA and/or 5 AAA1 gene. However, the various aspects and embodiments are also directed to other GPRA and/or AAA1 genes, such as homolog genes and transcript variants, and polymorphisms (e.g., single nucleotide polymorphism, (SNPs)) associated with certain GPRA and/or AAA1 genes. As such, the various aspects and embodiments are also directed to other genes that are involved in GPRA and/or AAA1 mediated pathways of 10 signal transduction or gene expression that are involved, for example, in the the maintenance or development of diseases, traits, or conditions described herein. These additional genes can be analyzed for target sites using the methods described for GPRA and/or AAA1 genes herein. Thus, the modulation of other genes and the effects of such modulation of the other genes can be performed, determined, and measured as described 15 herein.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a GPRA and/or AAA1 gene, wherein said siNA molecule comprises about 15 to about 28 base pairs.

In one embodiment, the invention features a double stranded short interfering 20 nucleic acid (siNA) molecule that directs cleavage of a GPRA and/or AAA1 RNA via RNA interference (RNAi), wherein the double stranded siNA molecule comprises a first and a second strand, each strand of the siNA molecule is about 18 to about 28 nucleotides in length, the first strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the GPRA and/or AAA1 RNA for the 25 siNA molecule to direct cleavage of the GPRA and/or AAA1 RNA via RNA interference, and the second strand of said siNA molecule comprises nucleotide sequence that is complementary to the first strand.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a GPRA and/or AAA1 RNA via 30 RNA interference (RNAi), wherein the double stranded siNA molecule comprises a first and a second strand, each strand of the siNA molecule is about 18 to about 23

nucleotides in length, the first strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the GPRA and/or AAA1 RNA for the siNA molecule to direct cleavage of the GPRA and/or AAA1 RNA via RNA interference, and the second strand of said siNA molecule comprises nucleotide sequence 5 that is complementary to the first strand.

In one embodiment, the invention features a chemically synthesized double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a GPRA and/or AAA1 RNA via RNA interference (RNAi), wherein each strand of the siNA molecule is about 18 to about 28 nucleotides in length; and one strand of the siNA 10 molecule comprises nucleotide sequence having sufficient complementarity to the GPRA and/or AAA1 RNA for the siNA molecule to direct cleavage of the GPRA and/or AAA1 RNA via RNA interference.

In one embodiment, the invention features a chemically synthesized double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a GPRA and/or AAA1 RNA via RNA interference (RNAi), wherein each strand of the siNA 15 molecule is about 18 to about 23 nucleotides in length; and one strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the GPRA and/or AAA1 RNA for the siNA molecule to direct cleavage of the GPRA and/or AAA1 RNA via RNA interference.

20 In one embodiment, the invention features a siNA molecule that down-regulates expression of a GPRA and/or AAA1 gene, for example, wherein the GPRA and/or AAA1 gene comprises GPRA and/or AAA1 encoding sequence. In one embodiment, the invention features a siNA molecule that down-regulates expression of a GPRA and/or AAA1 gene, for example, wherein the GPRA and/or AAA1 gene comprises 25 GPRA and/or AAA1 non-coding sequence or regulatory elements involved in GPRA and/or AAA1 gene expression.

In one embodiment, a siNA of the invention is used to inhibit the expression of GPRA and/or AAA1 genes or a GPRA and/or AAA1 gene family (e.g., GPRA and/or AAA1 superfamily genes), wherein the genes or gene family sequences share sequence 30 homology. Such homologous sequences can be identified as is known in the art, for

example using sequence alignments. siNA molecules can be designed to target such homologous sequences, for example using perfectly complementary sequences or by incorporating non-canonical base pairs, for example mismatches and/or wobble base pairs, that can provide additional target sequences. In instances where mismatches are 5 identified, non-canonical base pairs (for example, mismatches and/or wobble bases) can be used to generate siNA molecules that target more than one gene sequence. In a non-limiting example, non-canonical base pairs such as UU and CC base pairs are used to generate siNA molecules that are capable of targeting sequences for differing GPRA and/or AAA1 targets that share sequence homology. As such, one advantage of using 10 siNAs of the invention is that a single siNA can be designed to include nucleic acid sequence that is complementary to the nucleotide sequence that is conserved between the homologous genes. In this approach, a single siNA can be used to inhibit expression of more than one gene instead of using more than one siNA molecule to target the different genes.

15        In one embodiment, the invention features a siNA molecule having RNAi activity against GPRA and/or AAA1 RNA, wherein the siNA molecule comprises a sequence complementary to any RNA having GPRA and/or AAA1 encoding sequence, such as those sequences having GenBank Accession Nos. shown in **Table I**. In another embodiment, the invention features a siNA molecule having RNAi activity against 20 GPRA and/or AAA1 RNA, wherein the siNA molecule comprises a sequence complementary to an RNA having variant GPRA and/or AAA1 encoding sequence, for example other mutant GPRA and/or AAA1 genes not shown in **Table I** but known in the art to be associated with the maintenance and/or development of inflammatory and/or respiratory diseases, disorders, and/or conditions. Chemical modifications as shown in 25 **Tables III and IV** or otherwise described herein can be applied to any siNA construct of the invention. In another embodiment, a siNA molecule of the invention includes a nucleotide sequence that can interact with nucleotide sequence of a GPRA and/or AAA1 gene and thereby mediate silencing of GPRA and/or AAA1 gene expression, for example, wherein the siNA mediates regulation of GPRA and/or AAA1 gene expression 30 by cellular processes that modulate the chromatin structure or methylation patterns of the GPRA and/or AAA1 gene and prevent transcription of the GPRA and/or AAA1 gene.

In one embodiment, siNA molecules of the invention are used to down regulate or inhibit the expression of GPRA and/or AAA1 proteins arising from GPRA and/or AAA1 haplotype polymorphisms that are associated with a disease or condition, (e.g., inflammatory and/or respiratory diseases, disorders, and/or conditions). Analysis of 5 GPRA and/or AAA1 genes, or GPRA and/or AAA1 protein or RNA levels can be used to identify subjects with such polymorphisms or those subjects who are at risk of developing traits, conditions, or diseases described herein. These subjects are amenable to treatment, for example, treatment with siNA molecules of the invention and any other composition useful in treating diseases related to GPRA and/or AAA1 gene expression.

10 As such, analysis of GPRA and/or AAA1 protein or RNA levels can be used to determine treatment type and the course of therapy in treating a subject. Monitoring of GPRA and/or AAA1 protein or RNA levels can be used to predict treatment outcome and to determine the efficacy of compounds and compositions that modulate the level and/or activity of certain GPRA and/or AAA1 proteins associated with a trait, condition, 15 or disease.

In one embodiment of the invention a siNA molecule comprises an antisense strand comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof encoding a GPRA and/or AAA1 protein. The siNA further comprises a sense strand, wherein said sense strand comprises a nucleotide sequence of a 20 GPRA and/or AAA1 gene or a portion thereof.

In another embodiment, a siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence encoding a GPRA and/or AAA1 protein or a portion thereof. The siNA molecule further comprises a sense region, wherein said sense region comprises a nucleotide sequence of a 25 GPRA and/or AAA1 gene or a portion thereof.

In another embodiment, the invention features a siNA molecule comprising a nucleotide sequence in the antisense region of the siNA molecule that is complementary to a nucleotide sequence or portion of sequence of a GPRA and/or AAA1 gene. In another embodiment, the invention features a siNA molecule comprising a region, for 30 example, the antisense region of the siNA construct, complementary to a sequence comprising a GPRA and/or AAA1 gene sequence or a portion thereof.

In one embodiment, the antisense region of GPRA siNA constructs comprises a sequence complementary to sequence having any of SEQ ID NOs. 1-87, 175-188, 581-588, 597-604, 613-620, 629-636, 645-652, 661-668, 789, 791, 793, 795, 796, 798, 800, 802, 804, or 805. In one embodiment, the antisense region of GPRA and/or AAA1 constructs comprises sequence having any of SEQ ID NOs. 88-174, 189-202, 605-612, 621-628, 637-644, 653-660, 669-692, 790, 792, 794, 797, 799, 801, 803, or 806. In another embodiment, the sense region of GPRA constructs comprises sequence having any of SEQ ID NOs. 1-87, 175-188, 581-588, 597-604, 613-620, 629-636, 645-652, 661-668, 789, 791, 793, 795, 796, 798, 800, 802, 804, or 805.

10 In one embodiment, a siNA molecule of the invention comprises any of SEQ ID NOs. 1-806. The sequences shown in SEQ ID NOs. 1-806 are not limiting. A siNA molecule of the invention can comprise any contiguous GPRA and/or AAA1 sequence (e.g., about 15 to about 25 or more, or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more contiguous GPRA and/or AAA1 nucleotides).

15 In yet another embodiment, the invention features a siNA molecule comprising a sequence, for example, the antisense sequence of the siNA construct, complementary to a sequence or portion of sequence comprising sequence represented by GenBank Accession Nos. shown in **Table I**. Chemical modifications in **Tables III and IV** and described herein can be applied to any siNA construct of the invention.

20 In one embodiment of the invention a siNA molecule comprises an antisense strand having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein the antisense strand is complementary to a RNA sequence or a portion thereof encoding a GPRA and/or AAA1 protein, and wherein said siNA further comprises a sense strand having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, and wherein said sense strand and said antisense strand are distinct nucleotide sequences where at least about 15 nucleotides in each strand are complementary to the other strand.

25 In another embodiment of the invention a siNA molecule of the invention comprises an antisense region having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein the antisense region is

complementary to a RNA sequence encoding a GPRA and/or AAA1 protein, and wherein said siNA further comprises a sense region having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein said sense region and said antisense region are comprised in a linear molecule  
5 where the sense region comprises at least about 15 nucleotides that are complementary to the antisense region.

In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a GPRA and/or AAA1 gene. Because GPRA and/or AAA1 (e.g., GPRA and/or AAA1 superfamily) genes can share some degree of  
10 sequence homology with each other, siNA molecules can be designed to target a class of GPRA and/or AAA1 genes or alternately specific GPRA and/or AAA1 genes (e.g., polymorphic variants) by selecting sequences that are either shared amongst different GPRA and/or AAA1 targets or alternatively that are unique for a specific GPRA and/or AAA1 target. Therefore, in one embodiment, the siNA molecule can be designed to target  
15 conserved regions of GPRA and/or AAA1 RNA sequences having homology among several GPRA and/or AAA1 gene variants so as to target a class of GPRA and/or AAA1 genes with one siNA molecule. Accordingly, in one embodiment, the siNA molecule of the invention modulates the expression of one or both GPRA and/or AAA1 alleles in a subject. In another embodiment, the siNA molecule can be designed to target  
20 a sequence that is unique to a specific GPRA and/or AAA1 RNA sequence (e.g., a single GPRA and/or AAA1 allele or GPRA and/or AAA1 single nucleotide polymorphism (SNP)) due to the high degree of specificity that the siNA molecule requires to mediate RNAi activity.

In one embodiment, nucleic acid molecules of the invention that act as mediators  
25 of the RNA interference gene silencing response are double-stranded nucleic acid molecules. In another embodiment, the siNA molecules of the invention consist of duplex nucleic acid molecules containing about 15 to about 30 base pairs between oligonucleotides comprising about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides. In yet another embodiment, siNA  
30 molecules of the invention comprise duplex nucleic acid molecules with overhanging ends of about 1 to about 3 (e.g., about 1, 2, or 3) nucleotides, for example, about 21-

nucleotide duplexes with about 19 base pairs and 3'-terminal mononucleotide, dinucleotide, or trinucleotide overhangs. In yet another embodiment, siNA molecules of the invention comprise duplex nucleic acid molecules with blunt ends, where both ends are blunt, or alternatively, where one of the ends is blunt.

5        In one embodiment, the invention features one or more chemically-modified siNA constructs having specificity for GPRA and/or AAA1 expressing nucleic acid molecules, such as RNA encoding a GPRA and/or AAA1 protein. In one embodiment, the invention features a RNA based siNA molecule (e.g., a siNA comprising 2'-OH nucleotides) having specificity for GPRA and/or AAA1 expressing nucleic acid 10 molecules that includes one or more chemical modifications described herein. Non-limiting examples of such chemical modifications include without limitation phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, "universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, and terminal glyceryl and/or inverted 15 deoxy abasic residue incorporation. These chemical modifications, when used in various siNA constructs, (e.g., RNA based siNA constructs), are shown to preserve RNAi activity in cells while at the same time, dramatically increasing the serum stability of these compounds. Furthermore, contrary to the data published by Parrish *et al.*, *supra*, applicant demonstrates that multiple (greater than one) phosphorothioate substitutions 20 are well-tolerated and confer substantial increases in serum stability for modified siNA constructs.

25        In one embodiment, a siNA molecule of the invention comprises modified nucleotides while maintaining the ability to mediate RNAi. The modified nucleotides can be used to improve *in vitro* or *in vivo* characteristics such as stability, activity, and/or bioavailability. For example, a siNA molecule of the invention can comprise modified nucleotides as a percentage of the total number of nucleotides present in the siNA molecule. As such, a siNA molecule of the invention can generally comprise about 5% to about 100% modified nucleotides (e.g., about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified 30 nucleotides). The actual percentage of modified nucleotides present in a given siNA molecule will depend on the total number of nucleotides present in the siNA. If the siNA

molecule is single stranded, the percent modification can be based upon the total number of nucleotides present in the single stranded siNA molecules. Likewise, if the siNA molecule is double stranded, the percent modification can be based upon the total number of nucleotides present in the sense strand, antisense strand, or both the sense and

5 antisense strands.

One aspect of the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a GPRA and/or AAA1 gene. In one embodiment, the double stranded siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is about 21 nucleotides long.

10 In one embodiment, the double-stranded siNA molecule does not contain any ribonucleotides. In another embodiment, the double-stranded siNA molecule comprises one or more ribonucleotides. In one embodiment, each strand of the double-stranded siNA molecule independently comprises about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein each strand

15 comprises about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to the nucleotides of the other strand. In one embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the GPRA and/or AAA1 gene, and the second strand of the double-

20 stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence of the GPRA and/or AAA1 gene or a portion thereof.

In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a GPRA and/or AAA1 gene comprising an antisense region, wherein the antisense region comprises a

25 nucleotide sequence that is complementary to a nucleotide sequence of the GPRA and/or AAA1 gene or a portion thereof, and a sense region, wherein the sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence of the GPRA and/or AAA1 gene or a portion thereof. In one embodiment, the antisense region and the sense region independently comprise about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, wherein the antisense region

30

comprises about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to nucleotides of the sense region.

In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a GPRA and/or AAA1 5 gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the GPRA and/or AAA1 gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region.

In one embodiment, a siNA molecule of the invention comprises blunt ends, i.e., 10 ends that do not include any overhanging nucleotides. For example, a siNA molecule comprising modifications described herein (e.g., comprising nucleotides having Formulae I-VII or siNA constructs comprising “Stab 00”-“Stab 32” (Table IV) or any combination thereof (see Table IV)) and/or any length described herein can comprise blunt ends or ends with no overhanging nucleotides.

15 In one embodiment, any siNA molecule of the invention can comprise one or more blunt ends, i.e. where a blunt end does not have any overhanging nucleotides. In one embodiment, the blunt ended siNA molecule has a number of base pairs equal to the number of nucleotides present in each strand of the siNA molecule. In another embodiment, the siNA molecule comprises one blunt end, for example wherein the 5'- 20 end of the antisense strand and the 3'-end of the sense strand do not have any overhanging nucleotides. In another example, the siNA molecule comprises one blunt end, for example wherein the 3'-end of the antisense strand and the 5'-end of the sense strand do not have any overhanging nucleotides. In another example, a siNA molecule comprises two blunt ends, for example wherein the 3'-end of the antisense strand and the 25 5'-end of the sense strand as well as the 5'-end of the antisense strand and 3'-end of the sense strand do not have any overhanging nucleotides. A blunt ended siNA molecule can comprise, for example, from about 15 to about 30 nucleotides (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides). Other nucleotides present in a blunt ended siNA molecule can comprise, for example, mismatches, bulges, 30 loops, or wobble base pairs to modulate the activity of the siNA molecule to mediate RNA interference.

By "blunt ends" is meant symmetric termini or termini of a double stranded siNA molecule having no overhanging nucleotides. The two strands of a double stranded siNA molecule align with each other without over-hanging nucleotides at the termini. For example, a blunt ended siNA construct comprises terminal nucleotides that are 5 complementary between the sense and antisense regions of the siNA molecule.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a GPRA and/or AAA1 gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment 10 comprises the antisense region of the siNA molecule. The sense region can be connected to the antisense region via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker.

In one embodiment, the invention features double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a GPRA and/or AAA1 gene, wherein the siNA molecule comprises about 15 to about 30 (e.g. about 15, 16, 17, 15 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein each strand of the siNA molecule comprises one or more chemical modifications. In another embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of a GPRA and/or 20 AAA1 gene or a portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of the GPRA and/or AAA1 gene. In another embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of a GPRA and/or AAA1 gene 25 or portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or portion thereof of the GPRA and/or AAA1 gene. In another embodiment, each strand of the siNA molecule comprises about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, and each strand comprises at least about 30 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30)

nucleotides that are complementary to the nucleotides of the other strand. The GPRA and/or AAA1 gene can comprise, for example, sequences referred to in **Table I**.

In one embodiment, a siNA molecule of the invention comprises no ribonucleotides. In another embodiment, a siNA molecule of the invention comprises 5 ribonucleotides.

In one embodiment, a siNA molecule of the invention comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence of a GPRA and/or AAA1 gene or a portion thereof, and the siNA further comprises a sense region comprising a nucleotide sequence substantially similar to the nucleotide 10 sequence of the GPRA and/or AAA1 gene or a portion thereof. In another embodiment, the antisense region and the sense region each comprise about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides and the antisense region comprises at least about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to 15 nucleotides of the sense region. The GPRA and/or AAA1 gene can comprise, for example, sequences referred to in **Table I**. In another embodiment, the siNA is a double stranded nucleic acid molecule, where each of the two strands of the siNA molecule independently comprise about 15 to about 40 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 23, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides, and 20 where one of the strands of the siNA molecule comprises at least about 15 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 or more) nucleotides that are complementary to the nucleic acid sequence of the GPRA and/or AAA1 gene or a portion thereof.

In one embodiment, a siNA molecule of the invention comprises a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence 25 that is complementary to a nucleotide sequence of RNA encoded by a GPRA and/or AAA1 gene, or a portion thereof, and the sense region comprises a nucleotide sequence that is complementary to the antisense region. In one embodiment, the siNA molecule is assembled from two separate oligonucleotide fragments, wherein one fragment comprises the sense region and the second fragment comprises the antisense region of 30 the siNA molecule. In another embodiment, the sense region is connected to the antisense region via a linker molecule. In another embodiment, the sense region is

connected to the antisense region via a linker molecule, such as a nucleotide or non-nucleotide linker. The GPRA and/or AAA1 gene can comprise, for example, sequences referred in to **Table I**.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a GPRA and/or AAA1 gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the GPRA and/or AAA1 gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the siNA molecule has one or more modified pyrimidine and/or purine nucleotides. In one embodiment, the pyrimidine nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides or 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In another embodiment, the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In another embodiment, the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In one embodiment, the pyrimidine nucleotides in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the antisense region are 2'-O-methyl or 2'-deoxy purine nucleotides. In another embodiment of any of the above-described siNA molecules, any nucleotides present in a non-complementary region of the sense strand (e.g. overhang region) are 2'-deoxy nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a GPRA and/or AAA1 gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule, and wherein the fragment comprising the sense region includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the fragment. In one embodiment, the terminal cap moiety is an inverted deoxy abasic moiety or glyceryl moiety. In one embodiment, each of the two

fragments of the siNA molecule independently comprise about 15 to about 30 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides. In another embodiment, each of the two fragments of the siNA molecule independently comprise about 15 to about 40 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 5 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides. In a non-limiting example, each of the two fragments of the siNA molecule comprise about 21 nucleotides.

In one embodiment, the invention features a siNA molecule comprising at least one modified nucleotide, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide. The siNA can be, for example, about 15 to about 40 nucleotides in length. In one 10 embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In one embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'-deoxy-2'-fluoro uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro uridine nucleotides. In one 15 embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In one embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides. In one embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine 20 nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as phosphorothioate linkage. In one embodiment, the 2'-deoxy-2'-fluoronucleotides are present at specifically selected locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides.

In one embodiment, the invention features a method of increasing the stability of a 25 siNA molecule against cleavage by ribonucleases comprising introducing at least one modified nucleotide into the siNA molecule, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide. In one embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In one embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'- 30 deoxy-2'-fluoro uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro

uridine nucleotides. In one embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In one embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides.

5 In one embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as phosphorothioate linkage. In one embodiment, the 2'-deoxy-2'-fluoronucleotides are present at specifically selected locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine

10 nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a GPRA and/or AAA1 gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the GPRA and/or AAA1 gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the purine nucleotides present in the antisense region comprise 2'-deoxy- purine nucleotides. In an alternative embodiment, the purine nucleotides present in the antisense region comprise 2'-O-methyl purine nucleotides. In either of the above embodiments, the antisense region can comprise a phosphorothioate internucleotide linkage at the 3' end of the antisense region. Alternatively, in either of the above embodiments, the antisense region can comprise a glyceryl modification at the 3' end of the antisense region. In another embodiment of any of the above-described siNA molecules, any nucleotides present in a non-complementary region of the antisense 25 strand (e.g. overhang region) are 2'-deoxy nucleotides.

In one embodiment, the antisense region of a siNA molecule of the invention comprises sequence complementary to a portion of a GPRA and/or AAA1 transcript having sequence unique to a particular GPRA and/or AAA1 disease related allele, such as sequence comprising a single nucleotide polymorphism (SNP) associated with the 30 disease specific allele. As such, the antisense region of a siNA molecule of the invention can comprise sequence complementary to sequences that are unique to a particular allele

to provide specificity in mediating selective RNAi against the disease, condition, or trait related allele.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a GPRA and/or AAA1 gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule, where each strand is about 21 nucleotides long and where about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule, wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule, where each strand is about 19 nucleotide long and where the nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule to form at least about 15 (e.g., 15, 16, 17, 18, or 19) base pairs, wherein one or both ends of the siNA molecule are blunt ends. In one embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine nucleotide, such as a 2'-deoxy-thymidine. In another embodiment, all nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule of about 19 to about 25 base pairs having a sense region and an antisense region, where about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the GPRA and/or AAA1 gene. In another embodiment, about 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the GPRA and/or AAA1 gene. In any of the above embodiments, the 5'-end of the fragment comprising said antisense region can optionally include a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a GPRA and/or AAA1 RNA

sequence (e.g., wherein said target RNA sequence is encoded by a GPRA and/or AAA1 gene involved in the GPRA and/or AAA1 pathway), wherein the siNA molecule does not contain any ribonucleotides and wherein each strand of the double-stranded siNA molecule is about 15 to about 30 nucleotides. In one embodiment, the siNA molecule is 5 21 nucleotides in length. Examples of non-ribonucleotide containing siNA constructs are combinations of stabilization chemistries shown in **Table IV** in any combination of Sense/Antisense chemistries, such as Stab 7/8, Stab 7/11, Stab 8/8, Stab 18/8, Stab 18/11, Stab 12/13, Stab 7/13, Stab 18/13, Stab 7/19, Stab 8/19, Stab 18/19, Stab 7/20, Stab 8/20, Stab 18/20, Stab 7/32, Stab 8/32, or Stab 18/32 (e.g., any siNA having Stab 7, 8, 11, 12, 10 13, 14, 15, 17, 18, 19, 20, or 32 sense or antisense strands or any combination thereof).

In one embodiment, the invention features a chemically synthesized double stranded RNA molecule that directs cleavage of a GPRA and/or AAA1 RNA via RNA interference, wherein each strand of said RNA molecule is about 15 to about 30 nucleotides in length; one strand of the RNA molecule comprises nucleotide sequence 15 having sufficient complementarity to the GPRA and/or AAA1 RNA for the RNA molecule to direct cleavage of the GPRA and/or AAA1 RNA via RNA interference; and wherein at least one strand of the RNA molecule optionally comprises one or more chemically modified nucleotides described herein, such as without limitation deoxynucleotides, 2'-O-methyl nucleotides, 2'-deoxy-2'-fluoro nucleotides, 2'-O- 20 methoxyethyl nucleotides etc.

In one embodiment, the invention features a medicament comprising a siNA molecule of the invention.

In one embodiment, the invention features an active ingredient comprising a siNA molecule of the invention.

25 In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule to inhibit, down-regulate, or reduce expression of a GPRA and/or AAA1 gene, wherein the siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is independently about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 30 27, 28, 29 or 30 or more) nucleotides long. In one embodiment, the siNA molecule of

the invention is a double stranded nucleic acid molecule comprising one or more chemical modifications, where each of the two fragments of the siNA molecule independently comprise about 15 to about 40 (e.g. about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 23, 33, 34, 35, 36, 37, 38, 39, or 40) nucleotides and

5 where one of the strands comprises at least 15 nucleotides that are complementary to nucleotide sequence of GPRA and/or AAA1 encoding RNA or a portion thereof. In a non-limiting example, each of the two fragments of the siNA molecule comprise about 21 nucleotides. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule comprising one or more chemical modifications, where each strand is

10 about 21 nucleotide long and where about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule, wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid

15 molecule comprising one or more chemical modifications, where each strand is about 19 nucleotide long and where the nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule to form at least about 15 (e.g., 15, 16, 17, 18, or 19) base pairs, wherein one or both ends of the siNA molecule are blunt ends. In one embodiment, each of the two 3'

20 terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine nucleotide, such as a 2'-deoxy-thymidine. In another embodiment, all nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule. In another embodiment, the siNA molecule is a double stranded nucleic acid molecule of about 19 to about 25 base pairs having a

25 sense region and an antisense region and comprising one or more chemical modifications, where about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the GPRA and/or AAA1 gene. In another embodiment, about 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the

30 GPRA and/or AAA1 gene. In any of the above embodiments, the 5'-end of the fragment comprising said antisense region can optionally include a phosphate group.

In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits, down-regulates, or reduces expression of a GPRA and/or AAA1 gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that

5 is complementary to nucleotide sequence of GPRA and/or AAA1 RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

10 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits, down-regulates, or reduces expression of a GPRA and/or AAA1 gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of GPRA and/or AAA1 RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

20 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits, down-regulates, or reduces expression of a GPRA and/or AAA1 gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of GPRA and/or AAA1 RNA that encodes a protein or portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, each strand of the siNA molecule comprises about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 25 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides, wherein each strand comprises 30 at least about 15 nucleotides that are complementary to the nucleotides of the other strand. In one embodiment, the siNA molecule is assembled from two oligonucleotide

fragments, wherein one fragment comprises the nucleotide sequence of the antisense strand of the siNA molecule and a second fragment comprises nucleotide sequence of the sense region of the siNA molecule. In one embodiment, the sense strand is connected to the antisense strand via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker. In a further embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In still another embodiment, the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-deoxy purine nucleotides. In another embodiment, the antisense strand comprises one or more 2'-deoxy-2'-fluoro pyrimidine nucleotides and one or more 2'-O-methyl purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-O-methyl purine nucleotides. In a further embodiment the sense strand comprises a 3'-end and a 5'-end, wherein a terminal cap moiety (e.g., an inverted deoxy abasic moiety or inverted deoxy nucleotide moiety such as inverted thymidine) is present at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the sense strand. In another embodiment, the antisense strand comprises a phosphorothioate internucleotide linkage at the 3' end of the antisense strand. In another embodiment, the antisense strand comprises a glyceryl modification at the 3' end. In another embodiment, the 5'-end of the antisense strand optionally includes a phosphate group.

In any of the above-described embodiments of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a GPRA and/or AAA1 gene, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, each of the two strands of the siNA molecule can comprise about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides. In one embodiment, about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides of each strand of the siNA molecule are base-paired to the

complementary nucleotides of the other strand of the siNA molecule. In another embodiment, about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule, 5 wherein at least two 3' terminal nucleotides of each strand of the siNA molecule are not base-paired to the nucleotides of the other strand of the siNA molecule. In another embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine, such as 2'-deoxy-thymidine. In one embodiment, each strand of the siNA molecule is base-paired to the complementary nucleotides of the 10 other strand of the siNA molecule. In one embodiment, about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides of the antisense strand are base-paired to the nucleotide sequence of the GPRA and/or AAA1 RNA or a portion thereof. In one embodiment, about 18 to about 25 (e.g., about 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides of the antisense strand are base-paired to the 15 nucleotide sequence of the GPRA and/or AAA1 RNA or a portion thereof.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a GPRA and/or AAA1 gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of 20 GPRA and/or AAA1 RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the 5'-end of the antisense strand optionally includes a phosphate group.

25 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a GPRA and/or AAA1 gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of GPRA and/or AAA1 RNA or a portion thereof, the other strand is a sense strand which 30 comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the

double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence or a portion thereof of the antisense strand is complementary to a nucleotide sequence of the untranslated region or a portion thereof of the GPRA and/or AAA1 RNA.

5        In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a GPRA and/or AAA1 gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of GPRA and/or AAA1 RNA or a portion thereof, wherein the other strand is a sense strand  
10      which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence of the antisense strand is complementary to a nucleotide sequence of the GPRA and/or AAA1 RNA or a portion thereof that is present in the GPRA and/or  
15      AAA1 RNA.

In one embodiment, the invention features a composition comprising a siNA molecule of the invention in a pharmaceutically acceptable carrier or diluent.

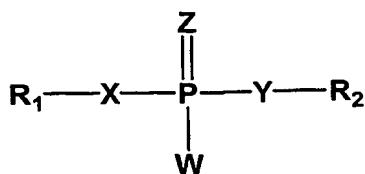
In a non-limiting example, the introduction of chemically-modified nucleotides into nucleic acid molecules provides a powerful tool in overcoming potential limitations  
20      of *in vivo* stability and bioavailability inherent to native RNA molecules that are delivered exogenously. For example, the use of chemically-modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given therapeutic effect since chemically-modified nucleic acid molecules tend to have a longer half-life in serum. Furthermore, certain chemical modifications can improve the  
25      bioavailability of nucleic acid molecules by targeting particular cells or tissues and/or improving cellular uptake of the nucleic acid molecule. Therefore, even if the activity of a chemically-modified nucleic acid molecule is reduced as compared to a native nucleic acid molecule, for example, when compared to an all-RNA nucleic acid molecule, the overall activity of the modified nucleic acid molecule can be greater than that of the  
30      native molecule due to improved stability and/or delivery of the molecule. Unlike native

unmodified siNA, chemically-modified siNA can also minimize the possibility of activating interferon activity in humans.

In any of the embodiments of siNA molecules described herein, the antisense region of a siNA molecule of the invention can comprise a phosphorothioate 5 internucleotide linkage at the 3'-end of said antisense region. In any of the embodiments of siNA molecules described herein, the antisense region can comprise about one to about five phosphorothioate internucleotide linkages at the 5'-end of said antisense region. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs of a siNA molecule of the invention can comprise ribonucleotides 10 or deoxyribonucleotides that are chemically-modified at a nucleic acid sugar, base, or backbone. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs can comprise one or more universal base ribonucleotides. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs can comprise one or more acyclic nucleotides.

15 One embodiment of the invention provides an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention in a manner that allows expression of the nucleic acid molecule. Another embodiment of the invention provides a mammalian cell comprising such an expression vector. The mammalian cell can be a human cell. The siNA molecule of the expression vector can 20 comprise a sense region and an antisense region. The antisense region can comprise sequence complementary to a RNA or DNA sequence encoding GPRA and/or AAA1 and the sense region can comprise sequence complementary to the antisense region. The siNA molecule can comprise two distinct strands having complementary sense and antisense regions. The siNA molecule can comprise a single strand having 25 complementary sense and antisense regions.

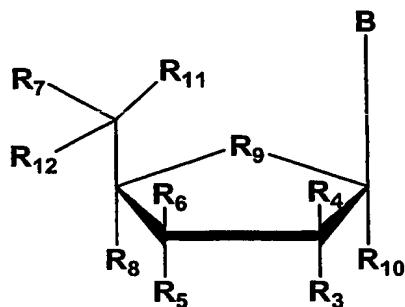
In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against GPRA and/or AAA1 inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) 30 nucleotides comprising a backbone modified internucleotide linkage having Formula I:



wherein each R<sub>1</sub> and R<sub>2</sub> is independently any nucleotide, non-nucleotide, or polynucleotide which can be naturally-occurring or chemically-modified, each X and Y is independently O, S, N, alkyl, or substituted alkyl, each Z and W is independently O, S, 5 N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, or acetyl and wherein W, X, Y, and Z are optionally not all O. In another embodiment, a backbone modification of the invention comprises a phosphonoacetate and/or thiophosphonoacetate internucleotide linkage (see for example Sheehan et al., 2003, Nucleic Acids Research, 31, 4109-4118).

The chemically-modified internucleotide linkages having Formula I, for example, 10 wherein any Z, W, X, and/or Y independently comprises a sulphur atom, can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) chemically-modified internucleotide linkages having Formula I at the 3'-end, the 5'-end, or both of 15 the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified internucleotide linkages having Formula I at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can 20 comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine nucleotides with chemically-modified 25 internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In another embodiment, a siNA molecule of the invention having internucleotide linkage(s) of Formula I also comprises a chemically-modified nucleotide or non-nucleotide having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against GPRA and/or AAA1 inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) 5 nucleotides or non-nucleotides having Formula II:

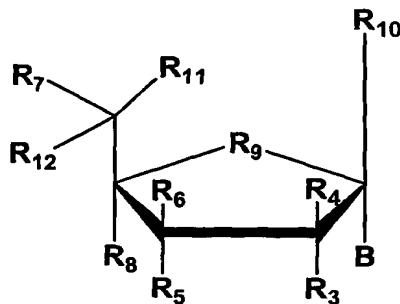


wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF<sub>3</sub>, OCF<sub>3</sub>, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-O SH, alkyl-OH, O-alkyl-OH, 10 O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO<sub>2</sub>, NO<sub>2</sub>, N<sub>3</sub>, NH<sub>2</sub>, aminoalkyl, aminoacid, aminoacyl, ONH<sub>2</sub>, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH<sub>2</sub>, S=O, CHF, or CF<sub>2</sub>, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-15 aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

20 The chemically-modified nucleotide or non-nucleotide of Formula II can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotides or non-nucleotides of Formula II at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the 25 antisense strand, or both strands. For example, an exemplary siNA molecule of the

invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 3'-end of the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against GPRA and/or AAA1 inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula III:

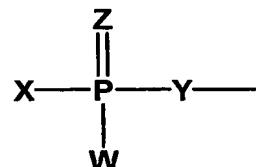


wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF<sub>3</sub>, OCF<sub>3</sub>, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO<sub>2</sub>, NO<sub>2</sub>, N<sub>3</sub>, NH<sub>2</sub>, aminoalkyl, aminoacid, aminoacyl, ONH<sub>2</sub>, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH<sub>2</sub>, S=O, CHF, or CF<sub>2</sub>, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be employed to be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula III can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotides or non-nucleotides of Formula III at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide(s) or non-nucleotide(s) of Formula III at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end of the sense strand, the antisense strand, or both strands.

In another embodiment, a siNA molecule of the invention comprises a nucleotide having Formula II or III, wherein the nucleotide having Formula II or III is in an inverted configuration. For example, the nucleotide having Formula II or III is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against GPRA and/or AAA1 inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a 5'-terminal phosphate group having Formula IV:



wherein each X and Y is independently O, S, N, alkyl, substituted alkyl, or alkylhalo; wherein each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, alkylhalo, or acetyl; and wherein W, X, Y and Z are not all O.

In one embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand, for example, a

strand complementary to a target RNA, wherein the siNA molecule comprises an all RNA siNA molecule. In another embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand wherein the siNA molecule also comprises about 1 to about 3 (e.g., about 1, 2, or 5 3) nucleotide 3'-terminal nucleotide overhangs having about 1 to about 4 (e.g., about 1, 2, 3, or 4) deoxyribonucleotides on the 3'-end of one or both strands. In another embodiment, a 5'-terminal phosphate group having Formula IV is present on the target-complementary strand of a siNA molecule of the invention, for example a siNA molecule having chemical modifications having any of Formulae I-VII.

10 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against GPRA and/or AAA1 inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more phosphorothioate internucleotide linkages. For example, in a non-limiting example, the invention features a chemically-modified short interfering nucleic acid (siNA) having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in one siNA strand. In yet another embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) individually having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in both siNA strands. The phosphorothioate internucleotide linkages can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can 15 comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) consecutive phosphorothioate internucleotide linkages at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine phosphorothioate internucleotide linkages in the sense strand, the 20 antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 25 30

8, 9, 10, or more) purine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a siNA molecule, wherein the sense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or about one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the sense strand comprises about 1 to about 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends

of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5 or more, for example about 1, 2, 3, 4, 5, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a siNA molecule, wherein the antisense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more 10 phosphorothioate internucleotide linkages, and/or about one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and 15 optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate 20 internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the 25 antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, 30 the 5'-end, or both of the 3' and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more 30 (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends

of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 5 universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or 10 without about 1 to about 5, for example about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule having about 1 to about 5 or more (specifically about 1, 2, 3, 4, 5 or more) phosphorothioate internucleotide linkages in each strand of the siNA molecule.

In another embodiment, the invention features a siNA molecule comprising 2'-5' internucleotide linkages. The 2'-5' internucleotide linkage(s) can be at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of one or both siNA sequence strands. In addition, the 20 2'-5' internucleotide linkage(s) can be present at various other positions within one or both siNA sequence strands, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a pyrimidine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a purine nucleotide in 25 one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage.

In another embodiment, a chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified, wherein each strand is independently about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length, wherein the 30 duplex has about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein the chemical modification comprises a

structure having any of Formulae I-VII. For example, an exemplary chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein each strand consists of about 21 5 nucleotides, each having a 2-nucleotide 3'-terminal nucleotide overhang, and wherein the duplex has about 19 base pairs. In another embodiment, a siNA molecule of the invention comprises a single stranded hairpin structure, wherein the siNA is about 36 to about 70 (e.g., about 36, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) 10 base pairs, and wherein the siNA can include a chemical modification comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 42 to about 50 (e.g., about 42, 43, 44, 45, 46, 47, 48, 49, or 15 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 19 to about 21 (e.g., 19, 20, or 21) base pairs and a 2-nucleotide 3'-terminal nucleotide overhang. In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. For example, a linear hairpin siNA molecule of the 20 invention is designed such that degradation of the loop portion of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In another embodiment, a siNA molecule of the invention comprises a hairpin structure, wherein the siNA is about 25 to about 50 (e.g., about 25, 26, 27, 28, 29, 30, 31, 25 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides in length having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs, and wherein the siNA can include one or 30 more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (e.g., about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination

thereof, wherein the linear oligonucleotide forms a hairpin structure having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In one embodiment, a linear hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

In another embodiment, a siNA molecule of the invention comprises an asymmetric hairpin structure, wherein the siNA is about 25 to about 50 (e.g., about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides in length having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (e.g., about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms an asymmetric hairpin structure having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In one embodiment, an asymmetric hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In another embodiment, an asymmetric hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

In another embodiment, a siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising sense and antisense regions, wherein the antisense region is about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in

length, wherein the sense region is about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides in length, wherein the sense region and the antisense region have at least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure

5 having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising sense and antisense regions, wherein the antisense region is about 18 to about 23 (e.g., about 18, 19, 20, 21, 22, or 23) nucleotides in length and wherein the sense region is about 3 to about 15 (e.g.,

10 about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) nucleotides in length, wherein the sense region and the antisense region have at least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. In another embodiment, the asymmetric double stranded siNA molecule can also have a 5'-terminal phosphate group that can be

15 chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV).

In another embodiment, a siNA molecule of the invention comprises a circular nucleic acid molecule, wherein the siNA is about 38 to about 70 (e.g., about 38, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) base pairs, and wherein the siNA can include a chemical modification, which comprises a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a circular oligonucleotide having about 42 to about 50 (e.g., about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is

20 chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the circular oligonucleotide forms a dumbbell shaped structure having about 19 base pairs and 2 loops.

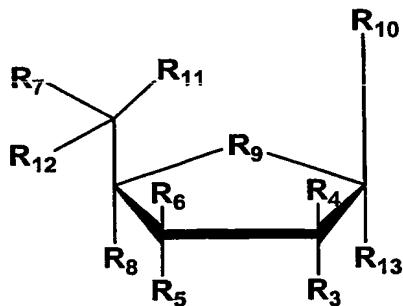
25

In another embodiment, a circular siNA molecule of the invention contains two loop motifs, wherein one or both loop portions of the siNA molecule is biodegradable.

30 For example, a circular siNA molecule of the invention is designed such that degradation of the loop portions of the siNA molecule *in vivo* can generate a double-stranded siNA

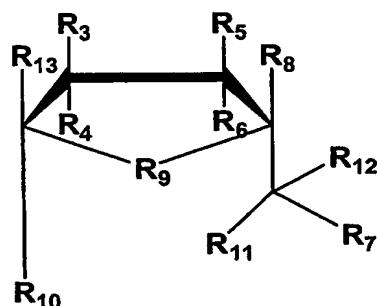
molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In one embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) abasic moiety, for example a compound having



wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF<sub>3</sub>, OCF<sub>3</sub>, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO<sub>2</sub>, NO<sub>2</sub>, N<sub>3</sub>, NH<sub>2</sub>, aminoalkyl, aminoacid, aminoacyl, ONH<sub>2</sub>, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH<sub>2</sub>, S=O, CHF, or CF<sub>2</sub>.

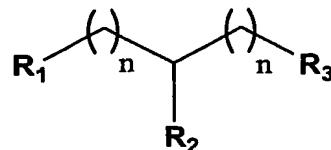
In one embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) inverted abasic moiety, for example a compound having Formula VI:



wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF<sub>3</sub>, OCF<sub>3</sub>, OCN, O-alkyl, S-

alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO<sub>2</sub>, NO<sub>2</sub>, N<sub>3</sub>, NH<sub>2</sub>, aminoalkyl, aminoacid, aminoacyl, ONH<sub>2</sub>, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, 5 substituted silyl, or group having Formula I or II; R<sub>9</sub> is O, S, CH<sub>2</sub>, S=O, CHF, or CF<sub>2</sub>, and either R<sub>2</sub>, R<sub>3</sub>, R<sub>8</sub> or R<sub>13</sub> serve as points of attachment to the siNA molecule of the invention.

In another embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) substituted polyalkyl moieties, for 10 example a compound having Formula VII:



wherein each n is independently an integer from 1 to 12, each R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF<sub>3</sub>, OCF<sub>3</sub>, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, 15 alkyl-OH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO<sub>2</sub>, NO<sub>2</sub>, N<sub>3</sub>, NH<sub>2</sub>, aminoalkyl, aminoacid, aminoacyl, ONH<sub>2</sub>, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or a group having Formula I, and R<sub>1</sub>, R<sub>2</sub> or R<sub>3</sub> serves as points of attachment to the siNA molecule of the invention.

20 In another embodiment, the invention features a compound having Formula VII, wherein R<sub>1</sub> and R<sub>2</sub> are hydroxyl (OH) groups, n = 1, and R<sub>3</sub> comprises O and is the point of attachment to the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both strands of a double-stranded siNA molecule of the invention or to a single-stranded siNA molecule of the invention. This modification is referred to herein as "glyceryl" (for 25 example modification 6 in Figure 10).

In another embodiment, a chemically modified nucleoside or non-nucleoside (e.g. a moiety having any of Formula V, VI or VII) of the invention is at the 3'-end, the 5'-end,

or both of the 3' and 5'-ends of a siNA molecule of the invention. For example, chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) can be present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense strand, the sense strand, or both antisense and sense strands of the siNA molecule. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the terminal 5 position of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the two terminal positions of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the penultimate position of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In addition, a moiety having Formula VII can be present at the 3'-end or the 5'-end of a hairpin siNA molecule as described herein.

In another embodiment, a siNA molecule of the invention comprises an abasic residue having Formula V or VI, wherein the abasic residue having Formula VI or VI is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, a siNA molecule of the invention comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) locked nucleic acid (LNA) nucleotides, for example, at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In another embodiment, a siNA molecule of the invention comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) acyclic nucleotides, for example, at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

10 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

30 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any

(e.g., one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), wherein any (e.g., one or more or all) purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

10 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

20 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), wherein any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said antisense region are 2'-deoxy nucleotides.

30 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein

any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or 5 more or all) purine nucleotides present in the antisense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein 10 any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine 15 nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against GPRA and/or AAA1 inside a cell or reconstituted *in vitro* system 20 comprising a sense region, wherein one or more pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and one or more purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (e.g., 25 wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and an antisense region, wherein one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy- 30 2'-fluoro pyrimidine nucleotides), and one or more purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides

are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). The sense region and/or the antisense region can have a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the 5 sense and/or antisense sequence. The sense and/or antisense region can optionally further comprise a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides. The overhang nucleotides can further comprise one or more (e.g., about 1, 2, 3, 4 or more) phosphorothioate, phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages. Non-limiting examples of these 10 chemically-modified siRNAs are shown in **Figures 4 and 5 and Tables III and IV** herein. In any of these described embodiments, the purine nucleotides present in the sense region are alternatively 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides) and one or more purine nucleotides 15 present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). Also, in any of these embodiments, one or more purine nucleotides present in the sense region are alternatively purine ribonucleotides (e.g., wherein all purine nucleotides are purine ribonucleotides or 20 alternately a plurality of purine nucleotides are purine ribonucleotides) and any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). Additionally, in any 25 of these embodiments, one or more purine nucleotides present in the sense region and/or present in the antisense region are alternatively selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides (e.g., wherein all purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides). 30

30

In another embodiment, any modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, are resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi. Non-limiting examples of nucleotides having a northern configuration include locked nucleic acid (LNA) nucleotides (e.g., 2'-O, 4'-C-methylene-(D-ribofuranosyl) nucleotides); 2'-methoxyethoxy (MOE) nucleotides; 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotides, 2'-deoxy-2'-chloro nucleotides, 2'-azido nucleotides, and 2'-O-methyl nucleotides.

In one embodiment, the sense strand of a double stranded siNA molecule of the invention comprises a terminal cap moiety, (see for example Figure 10) such as an inverted deoxyabasic moiety, at the 3'-end, 5'-end, or both 3' and 5'-ends of the sense strand.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid molecule (siNA) capable of mediating RNA interference (RNAi) against GPRA and/or AAA1 inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a conjugate covalently attached to the chemically-modified siNA molecule. Non-limiting examples of conjugates contemplated by the invention include conjugates and ligands described in Vargeese *et al.*, USSN 10/427,160, filed April 30, 2003, incorporated by reference herein in its entirety, including the drawings. In another embodiment, the conjugate is covalently attached to the chemically-modified siNA molecule via a biodegradable linker. In one embodiment, the conjugate molecule is attached at the 3'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In another embodiment, the conjugate

molecule is attached at the 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In yet another embodiment, the conjugate molecule is attached both the 3'-end and 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule, or any combination thereof. In one embodiment, a conjugate molecule of the invention comprises a molecule that facilitates delivery of a chemically-modified siNA molecule into a biological system, such as a cell. In another embodiment, the conjugate molecule attached to the chemically-modified siNA molecule is a polyethylene glycol, human serum albumin, or a ligand for a cellular receptor that can mediate cellular uptake.

10 Examples of specific conjugate molecules contemplated by the instant invention that can be attached to chemically-modified siNA molecules are described in Vargeese *et al.*, U.S. Serial No. 10/201,394, filed July 22, 2002 incorporated by reference herein. The type of conjugates used and the extent of conjugation of siNA molecules of the invention can be evaluated for improved pharmacokinetic profiles, bioavailability, and/or stability

15 of siNA constructs while at the same time maintaining the ability of the siNA to mediate RNAi activity. As such, one skilled in the art can screen siNA constructs that are modified with various conjugates to determine whether the siNA conjugate complex possesses improved properties while maintaining the ability to mediate RNAi, for example in animal models as are generally known in the art.

20 In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule of the invention, wherein the siNA further comprises a nucleotide, non-nucleotide, or mixed nucleotide/non-nucleotide linker that joins the sense region of the siNA to the antisense region of the siNA. In one embodiment, a nucleotide linker of the invention can be a linker of  $\geq 2$  nucleotides in length, for example about 3, 4, 5, 6, 7, 8, 25 9, or 10 nucleotides in length. In another embodiment, the nucleotide linker can be a nucleic acid aptamer. By “aptamer” or “nucleic acid aptamer” as used herein is meant a nucleic acid molecule that binds specifically to a target molecule wherein the nucleic acid molecule has sequence that comprises a sequence recognized by the target molecule in its natural setting. Alternately, an aptamer can be a nucleic acid molecule that binds to 30 a target molecule where the target molecule does not naturally bind to a nucleic acid. The target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand-binding domain of a protein, thereby preventing interaction of

the naturally occurring ligand with the protein. This is a non-limiting example and those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. (See, for example, Gold *et al.*, 1995, *Annu. Rev. Biochem.*, 64, 763; Brody and Gold, 2000, *J. Biotechnol.*, 74, 5; Sun, 2000, *Curr. Opin. Mol. Ther.*, 2, 100; Kusser, 2000, *J. Biotechnol.*, 74, 27; Hermann and Patel, 2000, *Science*, 287, 820; and Jayasena, 1999, *Clinical Chemistry*, 45, 1628.)

In yet another embodiment, a non-nucleotide linker of the invention comprises abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid, polyhydrocarbon, or other polymeric compounds (e.g. polyethylene glycols such as those having between 2 and 100 ethylene glycol units). Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* 1990, 18:6353 and *Nucleic Acids Res.* 1987, 15:3113; Cload and Schepartz, *J. Am. Chem. Soc.* 1991, 113:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* 1991, 113:5109; Ma *et al.*, *Nucleic Acids Res.* 1993, 21:2585 and *Biochemistry* 1993, 32:1751; Durand *et al.*, *Nucleic Acids Res.* 1990, 18:6353; McCurdy *et al.*, *Nucleosides & Nucleotides* 1991, 10:287; Jschke *et al.*, *Tetrahedron Lett.* 1993, 34:301; Ono *et al.*, *Biochemistry* 1991, 30:9914; Arnold *et al.*, International Publication No. WO 89/02439; Usman *et al.*, International Publication No. WO 95/06731; Dudycz *et al.*, International Publication No. WO 95/11910 and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, 113:4000, all hereby incorporated by reference herein. A "non-nucleotide" further means any group or compound that can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound can be abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine, for example at the C1 position of the sugar.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted *in vitro* system, wherein one or both strands of the siNA molecule that are assembled from two separate oligonucleotides do not comprise any ribonucleotides. For example, a siNA molecule can be assembled from a single oligonucleotide where the sense and antisense regions of the siNA comprise separate oligonucleotides that do not have any

ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotides. In another example, a siNA molecule can be assembled from a single oligonucleotide where the sense and antisense regions of the siNA are linked or circularized by a nucleotide or non-nucleotide linker as described herein, wherein the oligonucleotide does 5 not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotide. Applicant has surprisingly found that the presence of ribonucleotides (e.g., nucleotides having a 2'-hydroxyl group) within the siNA molecule is not required or essential to support RNAi activity. As such, in one embodiment, all positions within 10 the siNA can include chemically modified nucleotides and/or non-nucleotides such as nucleotides and/or non-nucleotides having Formula I, II, III, IV, V, VI, or VII or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted *in vitro* system 15 comprising a single stranded polynucleotide having complementarity to a target nucleic acid sequence. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group and a 3'-terminal phosphate group (e.g., a 2',3'-cyclic phosphate). In another embodiment, the 20 single stranded siNA molecule of the invention comprises about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides. In yet another embodiment, the single stranded siNA molecule of the invention comprises one or more chemically modified nucleotides or non-nucleotides described herein. For 25 example, all the positions within the siNA molecule can include chemically-modified nucleotides such as nucleotides having any of Formulae I-VII, or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted *in vitro* system 30 comprising a single stranded polynucleotide having complementarity to a target nucleic acid sequence, wherein one or more pyrimidine nucleotides present in the siNA are 2'-

deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., 5 wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence. The siNA optionally further comprises about 1 to about 4 or more (e.g., about 10 1, 2, 3, 4 or more) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, 4 or more) phosphorothioate, phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group. In any of these embodiments, any purine 15 nucleotides present in the antisense region are alternatively 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA (i.e., purine nucleotides present in the sense and/or antisense region) can alternatively be locked nucleic acid (LNA) 20 nucleotides (e.g., wherein all purine nucleotides are LNA nucleotides or alternately a plurality of purine nucleotides are LNA nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA are alternatively 2'-methoxyethyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-methoxyethyl purine nucleotides or alternately a plurality of purine nucleotides are 2'- 25 methoxyethyl purine nucleotides). In another embodiment, any modified nucleotides present in the single stranded siNA molecules of the invention comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for 30 example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the single stranded siNA molecules of

the invention are preferably resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi.

In one embodiment, a siNA molecule of the invention comprises chemically modified nucleotides or non-nucleotides (e.g., having any of Formulae I-VII, such as 2'-deoxy, 2'-deoxy-2'-fluoro, or 2'-O-methyl nucleotides) at alternating positions within one or more strands or regions of the siNA molecule. For example, such chemical modifications can be introduced at every other position of a RNA based siNA molecule, starting at either the first or second nucleotide from the 3'-end or 5'-end of the siNA. In a non-limiting example, a double stranded siNA molecule of the invention in which each strand of the siNA is 21 nucleotides in length is featured wherein positions 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21 of each strand are chemically modified (e.g., with compounds having any of Formulae 1-VII, such as such as 2'-deoxy, 2'-deoxy-2'-fluoro, or 2'-O-methyl nucleotides). In another non-limiting example, a double stranded siNA molecule of the invention in which each strand of the siNA is 21 nucleotides in length is featured wherein positions 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 of each strand are chemically modified (e.g., with compounds having any of Formulae 1-VII, such as such as 2'-deoxy, 2'-deoxy-2'-fluoro, or 2'-O-methyl nucleotides). Such siNA molecules can further comprise terminal cap moieties and/or backbone modifications as described herein.

In one embodiment, the invention features a method for modulating the expression of a GPRA and/or AAA1 gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the GPRA and/or AAA1 gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the GPRA and/or AAA1 gene in the cell.

In one embodiment, the invention features a method for modulating the expression of a GPRA and/or AAA1 gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the GPRA and/or AAA1 gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequence of the target RNA; and (b) introducing the siNA

molecule into a cell under conditions suitable to modulate the expression of the GPRA and/or AAA1 gene in the cell.

5 In another embodiment, the invention features a method for modulating the expression of more than one GPRA and/or AAA1 gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the GPRA and/or AAA1 genes; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the GPRA and/or AAA1 genes in the cell.

10 15 In another embodiment, the invention features a method for modulating the expression of two or more GPRA and/or AAA1 genes within a cell comprising: (a) synthesizing one or more siNA molecules of the invention, which can be chemically-modified, wherein the siNA strands comprise sequences complementary to RNA of the GPRA and/or AAA1 genes and wherein the sense strand sequences of the siNAs comprise sequences identical or substantially similar to the sequences of the target RNAs; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the GPRA and/or AAA1 genes in the cell.

20 25 In another embodiment, the invention features a method for modulating the expression of more than one GPRA and/or AAA1 gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the GPRA and/or AAA1 gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequences of the target RNAs; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the GPRA and/or AAA1 genes in the cell.

30 In one embodiment, siNA molecules of the invention are used as reagents in *ex vivo* applications. For example, siNA reagents are introduced into tissue or cells that are transplanted into a subject for therapeutic effect. The cells and/or tissue can be derived from an organism or subject that later receives the explant, or can be derived from another organism or subject prior to transplantation. The siNA molecules can be used to

modulate the expression of one or more genes in the cells or tissue, such that the cells or tissue obtain a desired phenotype or are able to perform a function when transplanted in vivo. In one embodiment, certain target cells from a patient are extracted. These extracted cells are contacted with siRNAs targeting a specific nucleotide sequence within 5 the cells under conditions suitable for uptake of the siRNAs by these cells (e.g. using delivery reagents such as cationic lipids, liposomes and the like or using techniques such as electroporation to facilitate the delivery of siRNAs into cells). The cells are then reintroduced back into the same patient or other patients. In one embodiment, the invention features a method of modulating the expression of a GPRA and/or AAA1 gene 10 in a tissue explant comprising: (a) synthesizing a siRNA molecule of the invention, which can be chemically-modified, wherein one of the siRNA strands comprises a sequence complementary to RNA of the GPRA and/or AAA1 gene; and (b) introducing the siRNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the GPRA and/or AAA1 gene in the 15 tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the GPRA and/or AAA1 gene in that organism.

In one embodiment, the invention features a method of modulating the expression 20 of a GPRA and/or AAA1 gene in a tissue explant comprising: (a) synthesizing a siRNA molecule of the invention, which can be chemically-modified, wherein one of the siRNA strands comprises a sequence complementary to RNA of the GPRA and/or AAA1 gene and wherein the sense strand sequence of the siRNA comprises a sequence identical or substantially similar to the sequence of the target RNA; and (b) introducing the siRNA 25 molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the GPRA and/or AAA1 gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the GPRA and/or 30 AAA1 gene in that organism.

In another embodiment, the invention features a method of modulating the expression of more than one GPRA and/or AAA1 gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the GPRA and/or AAA1 genes; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the GPRA and/or AAA1 genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the GPRA and/or AAA1 genes in that organism.

In one embodiment, the invention features a method of modulating the expression of a GPRA and/or AAA1 gene in a subject or organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the GPRA and/or AAA1 gene; and (b) introducing the siNA molecule into the subject or organism under conditions suitable to modulate the expression of the GPRA and/or AAA1 gene in the subject or organism. The level of GPRA and/or AAA1 protein or RNA can be determined using various methods well-known in the art.

In another embodiment, the invention features a method of modulating the expression of more than one GPRA and/or AAA1 gene in a subject or organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the GPRA and/or AAA1 genes; and (b) introducing the siNA molecules into the subject or organism under conditions suitable to modulate the expression of the GPRA and/or AAA1 genes in the subject or organism. The level of GPRA and/or AAA1 protein or RNA can be determined as is known in the art.

In one embodiment, the invention features a method for modulating the expression of a GPRA and/or AAA1 gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the GPRA

and/or AAA1 gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the GPRA and/or AAA1 gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one GPRA and/or AAA1 gene within a cell comprising: (a) 5 synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the GPRA and/or AAA1 gene; and (b) contacting the cell *in vitro* or *in vivo* with the siNA molecule under conditions suitable to modulate the expression of the GPRA and/or AAA1 genes in the cell.

10 In one embodiment, the invention features a method of modulating the expression of a GPRA and/or AAA1 gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the GPRA and/or AAA1 gene; and (b) contacting a cell of the tissue explant derived from a 15 particular subject or organism with the siNA molecule under conditions suitable to modulate the expression of the GPRA and/or AAA1 gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the subject or organism the tissue was derived from or into another subject or organism under conditions suitable to modulate the expression of the GPRA and/or 20 AAA1 gene in that subject or organism.

In another embodiment, the invention features a method of modulating the expression of more than one GPRA and/or AAA1 gene in a tissue explant comprising: (a) 25 synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the GPRA and/or AAA1 gene; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular subject or organism under conditions suitable to modulate the expression of the GPRA and/or AAA1 genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the subject or organism the tissue was derived from or into another subject or 30 organism under conditions suitable to modulate the expression of the GPRA and/or AAA1 genes in that subject or organism.

In one embodiment, the invention features a method of modulating the expression of a GPRA and/or AAA1 gene in a subject or organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the GPRA and/or AAA1 gene; and (b) introducing the siNA molecule into the subject or organism under conditions suitable to modulate the expression of the GPRA and/or AAA1 gene in the subject or organism.

In another embodiment, the invention features a method of modulating the expression of more than one GPRA and/or AAA1 gene in a subject or organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the GPRA and/or AAA1 gene; and (b) introducing the siNA molecules into the subject or organism under conditions suitable to modulate the expression of the GPRA and/or AAA1 genes in the subject or organism.

In one embodiment, the invention features a method of modulating the expression of a GPRA and/or AAA1 gene in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the GPRA and/or AAA1 gene in the subject or organism.

In one embodiment, the invention features a method for treating or preventing an inflammatory disease, disorder, or condition in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the GPRA and/or AAA1 gene in the subject or organism.

In one embodiment, the invention features a method for treating or preventing a respiratory disease, disorder, and/or condition in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the GPRA and/or AAA1 gene in the subject or organism.

In one embodiment, the invention features a method for treating or preventing asthma in a subject or organism comprising contacting the subject or organism with a

siNA molecule of the invention under conditions suitable to modulate the expression of the GPRA and/or AAA1 gene in the subject or organism.

In another embodiment, the invention features a method of modulating the expression of more than one GPRA and/or AAA1 genes in a subject or organism 5 comprising contacting the subject or organism with one or more siNA molecules of the invention under conditions suitable to modulate the expression of the GPRA and/or AAA1 genes in the subject or organism.

The siNA molecules of the invention can be designed to down regulate or inhibit target (e.g., GPRA and/or AAA1) gene expression through RNAi targeting of a variety 10 of RNA molecules. In one embodiment, the siNA molecules of the invention are used to target various RNAs corresponding to a target gene. Non-limiting examples of such RNAs include messenger RNA (mRNA), alternate RNA splice variants of target gene(s), post-transcriptionally modified RNA of target gene(s), pre-mRNA of target gene(s), and/or RNA templates. If alternate splicing produces a family of transcripts that are 15 distinguished by usage of appropriate exons, the instant invention can be used to inhibit gene expression through the appropriate exons to specifically inhibit or to distinguish among the functions of gene family members. For example, a protein that contains an alternatively spliced transmembrane domain can be expressed in both membrane bound and secreted forms. Use of the invention to target the exon containing the 20 transmembrane domain can be used to determine the functional consequences of pharmaceutical targeting of membrane bound as opposed to the secreted form of the protein. Non-limiting examples of applications of the invention relating to targeting these RNA molecules include therapeutic pharmaceutical applications, pharmaceutical discovery applications, molecular diagnostic and gene function applications, and gene 25 mapping, for example using single nucleotide polymorphism mapping with siNA molecules of the invention. Such applications can be implemented using known gene sequences or from partial sequences available from an expressed sequence tag (EST).

In another embodiment, the siNA molecules of the invention are used to target 30 conserved sequences corresponding to a gene family or gene families such as GPRA and/or AAA1 family genes. As such, siNA molecules targeting multiple GPRA and/or AAA1 targets can provide increased therapeutic effect. In addition, siNA can be used to

characterize pathways of gene function in a variety of applications. For example, the present invention can be used to inhibit the activity of target gene(s) in a pathway to determine the function of uncharacterized gene(s) in gene function analysis, mRNA function analysis, or translational analysis. The invention can be used to determine 5 potential target gene pathways involved in various diseases and conditions toward pharmaceutical development. The invention can be used to understand pathways of gene expression involved in, for example inflammatory and/or respiratory diseases, disorders and conditions.

10 In one embodiment, siNA molecule(s) and/or methods of the invention are used to down regulate the expression of gene(s) that encode RNA referred to by Genbank Accession, for example, GPRA and/or AAA1 genes encoding RNA sequence(s) referred to herein by Genbank Accession number, for example, Genbank Accession Nos. shown in **Table I**.

15 In one embodiment, the invention features a method comprising: (a) generating a library of siNA constructs having a predetermined complexity; and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target RNA sequence. In one embodiment, the siNA molecules of (a) have strands of a fixed length, for example, about 23 nucleotides in length. In another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 15 to 20 about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments 25 of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNase protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

30 In one embodiment, the invention features a method comprising: (a) generating a randomized library of siNA constructs having a predetermined complexity, such as of  $4^N$ , where N represents the number of base paired nucleotides in each of the siNA construct

strands (e.g. for a siNA construct having 21 nucleotide sense and antisense strands with 19 base pairs, the complexity would be  $4^{19}$ ); and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target GPRA and/or AAA1 RNA sequence. In another embodiment, the siNA molecules of (a) have 5 strands of a fixed length, for example about 23 nucleotides in length. In yet another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 10 27, 28, 29, or 30) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described in Example 6 herein. In another 15 embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of GPRA and/or AAA1 RNA are analyzed for detectable levels of cleavage, for example, by gel electrophoresis, northern blot analysis, or RNase protection assays, to determine the most suitable target site(s) within the target GPRA and/or AAA1 RNA sequence. The target GPRA and/or AAA1 RNA sequence can be obtained as is known in the art, for example, by cloning and/or 15 transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In another embodiment, the invention features a method comprising: (a) analyzing the sequence of a RNA target encoded by a target gene; (b) synthesizing one or more sets of siNA molecules having sequence complementary to one or more regions of the RNA 20 of (a); and (c) assaying the siNA molecules of (b) under conditions suitable to determine RNAi targets within the target RNA sequence. In one embodiment, the siNA molecules of (b) have strands of a fixed length, for example about 23 nucleotides in length. In another embodiment, the siNA molecules of (b) are of differing length, for example having strands of about 15 to about 30 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another 25 embodiment, the assay can comprise a cell culture system in which target RNA is expressed. Fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNase protection assays, to 30 determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by expression in *in vivo* systems.

By "target site" is meant a sequence within a target RNA that is "targeted" for cleavage mediated by a siNA construct which contains sequences within its antisense region that are complementary to the target sequence.

By "detectable level of cleavage" is meant cleavage of target RNA (and formation 5 of cleaved product RNAs) to an extent sufficient to discern cleavage products above the background of RNAs produced by random degradation of the target RNA. Production of cleavage products from 1-5% of the target RNA is sufficient to detect above the background for most methods of detection.

In one embodiment, the invention features a composition comprising a siNA 10 molecule of the invention, which can be chemically-modified, in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a pharmaceutical composition comprising siNA molecules of the invention, which can be chemically-modified, targeting one or more genes in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a method for 15 diagnosing a disease or condition in a subject comprising administering to the subject a composition of the invention under conditions suitable for the diagnosis of the disease or condition in the subject. In another embodiment, the invention features a method for treating or preventing a disease or condition in a subject, comprising administering to the subject a composition of the invention under conditions suitable for the treatment or 20 prevention of the disease or condition in the subject, alone or in conjunction with one or more other therapeutic compounds. In yet another embodiment, the invention features a method for treating or preventing inflammatory and/or respiratory diseases, disorders and 25 conditions in a subject or organism comprising administering to the subject a composition of the invention under conditions suitable for the treatment or prevention of inflammatory and/or respiratory diseases, disorders and conditions in the subject or organism.

In another embodiment, the invention features a method for validating a GPRA and/or AAA1 gene target, comprising: (a) synthesizing a siNA molecule of the 30 invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a GPRA and/or AAA1 target gene; (b) introducing the siNA molecule into a cell, tissue, subject, or organism under conditions

suitable for modulating expression of the GPRA and/or AAA1 target gene in the cell, tissue, subject, or organism; and (c) determining the function of the gene by assaying for any phenotypic change in the cell, tissue, subject, or organism.

In another embodiment, the invention features a method for validating a GPRA and/or AAA1 target comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a GPRA and/or AAA1 target gene; (b) introducing the siNA molecule into a biological system under conditions suitable for modulating expression of the GPRA and/or AAA1 target gene in the biological system; and (c) determining the function of the gene by assaying for any phenotypic change in the biological system.

By "biological system" is meant, material, in a purified or unpurified form, from biological sources, including but not limited to human or animal, wherein the system comprises the components required for RNAi activity. The term "biological system" includes, for example, a cell, tissue, subject, or organism, or extract thereof. The term biological system also includes reconstituted RNAi systems that can be used in an *in vitro* setting.

By "phenotypic change" is meant any detectable change to a cell that occurs in response to contact or treatment with a nucleic acid molecule of the invention (e.g., siNA). Such detectable changes include, but are not limited to, changes in shape, size, proliferation, motility, protein expression or RNA expression or other physical or chemical changes as can be assayed by methods known in the art. The detectable change can also include expression of reporter genes/molecules such as Green Fluorescent Protein (GFP) or various tags that are used to identify an expressed protein or any other cellular component that can be assayed.

In one embodiment, the invention features a kit containing a siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of a GPRA and/or AAA1 target gene in a biological system, including, for example, in a cell, tissue, subject, or organism. In another embodiment, the invention features a kit containing more than one siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of more than one

GPRA and/or AAA1 target gene in a biological system, including, for example, in a cell, tissue, subject, or organism.

In one embodiment, the invention features a cell containing one or more siNA molecules of the invention, which can be chemically-modified. In another embodiment, 5 the cell containing a siNA molecule of the invention is a mammalian cell. In yet another embodiment, the cell containing a siNA molecule of the invention is a human cell.

In one embodiment, the synthesis of a siNA molecule of the invention, which can be chemically-modified, comprises: (a) synthesis of two complementary strands of the 10 siNA molecule; (b) annealing the two complementary strands together under conditions suitable to obtain a double-stranded siNA molecule. In another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase oligonucleotide synthesis. In yet another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase tandem oligonucleotide synthesis.

In one embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing a first oligonucleotide sequence strand of the siNA molecule, wherein the first oligonucleotide sequence strand comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of the second oligonucleotide sequence strand of the siNA; (b) synthesizing the second oligonucleotide sequence strand of siNA on the scaffold of the first oligonucleotide sequence strand, 20 wherein the second oligonucleotide sequence strand further comprises a chemical moiety than can be used to purify the siNA duplex; (c) cleaving the linker molecule of (a) under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex; and (d) purifying the siNA duplex utilizing the chemical moiety of the second oligonucleotide sequence strand. In one embodiment, cleavage of the linker 25 molecule in (c) above takes place during deprotection of the oligonucleotide, for example, under hydrolysis conditions using an alkylamine base such as methylamine. In one embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support 30 as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity as the solid support derivatized linker, such that

cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place concomitantly. In another embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group, which can be employed in a trityl-on synthesis strategy as 5 described herein. In yet another embodiment, the chemical moiety, such as a dimethoxytrityl group, is removed during purification, for example, using acidic conditions.

In a further embodiment, the method for siNA synthesis is a solution phase synthesis or hybrid phase synthesis wherein both strands of the siNA duplex are 10 synthesized in tandem using a cleavable linker attached to the first sequence which acts a scaffold for synthesis of the second sequence. Cleavage of the linker under conditions suitable for hybridization of the separate siNA sequence strands results in formation of the double-stranded siNA molecule.

In another embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing one oligonucleotide sequence strand of the siNA molecule, wherein the sequence comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of another oligonucleotide sequence; (b) synthesizing a second oligonucleotide sequence having complementarity to the first sequence strand on the scaffold of (a), wherein the second sequence comprises the other 15 strand of the double-stranded siNA molecule and wherein the second sequence further comprises a chemical moiety than can be used to isolate the attached oligonucleotide sequence; (c) purifying the product of (b) utilizing the chemical moiety of the second oligonucleotide sequence strand under conditions suitable for isolating the full-length sequence comprising both siNA oligonucleotide strands connected by the cleavable 20 linker and under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example, under hydrolysis conditions. In another embodiment, cleavage of the linker molecule in (c) 25 above takes place after deprotection of the oligonucleotide. In another embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a 30

cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity or differing reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place 5 either concomitantly or sequentially. In one embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group.

In another embodiment, the invention features a method for making a double-stranded siNA molecule in a single synthetic process comprising: (a) synthesizing an 10 oligonucleotide having a first and a second sequence, wherein the first sequence is complementary to the second sequence, and the first oligonucleotide sequence is linked to the second sequence via a cleavable linker, and wherein a terminal 5'-protecting group, for example, a 5'-O-dimethoxytrityl group (5'-O-DMT) remains on the oligonucleotide having the second sequence; (b) deprotecting the oligonucleotide whereby the 15 deprotection results in the cleavage of the linker joining the two oligonucleotide sequences; and (c) purifying the product of (b) under conditions suitable for isolating the double-stranded siNA molecule, for example using a trityl-on synthesis strategy as described herein.

In another embodiment, the method of synthesis of siNA molecules of the 20 invention comprises the teachings of Scaringe *et al.*, US Patent Nos. 5,889,136; 6,008,400; and 6,111,086, incorporated by reference herein in their entirety.

In one embodiment, the invention features siNA constructs that mediate RNAi against GPRA and/or AAA1, wherein the siNA construct comprises one or more chemical modifications, for example, one or more chemical modifications having any of 25 Formulae I-VII or any combination thereof that increases the nuclease resistance of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules with increased nuclease resistance comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b)

assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased nuclease resistance.

In another embodiment, the invention features a method for generating siNA molecules with improved toxicologic profiles (e.g., have attenuated or no immunostimulatory properties) comprising (a) introducing nucleotides having any of Formula I-VII (e.g., siNA motifs referred to in **Table IV**) or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved toxicologic profiles.

In another embodiment, the invention features a method for generating siNA molecules that do not stimulate an interferon response (e.g., no interferon response or attenuated interferon response) in a cell, subject, or organism, comprising (a) introducing nucleotides having any of Formula I-VII (e.g., siNA motifs referred to in **Table IV**) or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules that do not stimulate an interferon response.

By "improved toxicologic profile", is meant that the chemically modified siNA construct exhibits decreased toxicity in a cell, subject, or organism compared to an unmodified siNA or siNA molecule having fewer modifications or modifications that are less effective in imparting improved toxicology. In a non-limiting example, siNA molecules with improved toxicologic profiles are associated with a decreased or attenuated immunostimulatory response in a cell, subject, or organism compared to an unmodified siNA or siNA molecule having fewer modifications or modifications that are less effective in imparting improved toxicology. In one embodiment, a siNA molecule with an improved toxicological profile comprises no ribonucleotides. In one embodiment, a siNA molecule with an improved toxicological profile comprises less than 5 ribonucleotides (e.g., 1, 2, 3, or 4 ribonucleotides). In one embodiment, a siNA molecule with an improved toxicological profile comprises Stab 7, Stab 8, Stab 11, Stab 12, Stab 13, Stab 16, Stab 17, Stab 18, Stab 19, Stab 20, Stab 23, Stab 24, Stab 25, Stab 26, Stab 27, Stab 28, Stab 29, Stab 30, Stab 31, Stab 32 or any combination thereof (see **Table IV**). In one embodiment, the level of immunostimulatory response associated with a given siNA molecule can be measured as is known in the art, for example by

determining the level of PKR/interferon response, proliferation, B-cell activation, and/or cytokine production in assays to quantitate the immunostimulatory response of particular siNA molecules (see, for example, Leifer *et al.*, 2003, *J Immunother.* 26, 313-9; and U.S. Patent No. 5968909, incorporated in its entirety by reference).

5 In one embodiment, the invention features siNA constructs that mediate RNAi against GPRA and/or AAA1, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the sense and antisense strands of the siNA construct.

10 In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the sense and antisense strands of the siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the sense and antisense strands of the siNA molecule.

15 In one embodiment, the invention features siNA constructs that mediate RNAi against GPRA and/or AAA1, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target RNA sequence within a cell.

20 In one embodiment, the invention features siNA constructs that mediate RNAi against GPRA and/or AAA1, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target DNA sequence within a cell.

25 In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for

isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence.

10 In one embodiment, the invention features siNA constructs that mediate RNAi against GPRA and/or AAA1, wherein the siNA construct comprises one or more chemical modifications described herein that modulate the polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA construct.

15 In another embodiment, the invention features a method for generating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to a chemically-modified siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying 20 the siNA molecule of step (a) under conditions suitable for isolating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA molecule.

25 In one embodiment, the invention features chemically-modified siNA constructs that mediate RNAi against GPRA and/or AAA1 in a cell, wherein the chemical modifications do not significantly effect the interaction of siNA with a target RNA molecule, DNA molecule and/or proteins or other factors that are essential for RNAi in a manner that would decrease the efficacy of RNAi mediated by such siNA constructs.

30 In another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against GPRA and/or AAA1 comprising (a)

introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity.

5 In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against GPRA and/or AAA1 target RNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target RNA.

10 In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against GPRA and/or AAA1 target DNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against 15 the target DNA.

In one embodiment, the invention features siNA constructs that mediate RNAi against GPRA and/or AAA1, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the cellular uptake of the siNA construct.

20 In another embodiment, the invention features a method for generating siNA molecules against GPRA and/or AAA1 with improved cellular uptake comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved cellular uptake.

25 In one embodiment, the invention features siNA constructs that mediate RNAi against GPRA and/or AAA1, wherein the siNA construct comprises one or more chemical modifications described herein that increases the bioavailability of the siNA construct, for example, by attaching polymeric conjugates such as polyethyleneglycol or equivalent conjugates that improve the pharmacokinetics of the siNA construct, or by 30 attaching conjugates that target specific tissue types or cell types *in vivo*. Non-limiting

examples of such conjugates are described in Vargeese *et al.*, U.S. Serial No. 10/201,394 incorporated by reference herein.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing a 5 conjugate into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such conjugates can include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and 10 other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; cholesterol; polyamines, such as spermine or spermidine; and others.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary 15 to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence is chemically modified in a manner that it can no longer act as a guide sequence for efficiently mediating RNA interference and/or be recognized by cellular proteins that facilitate RNAi.

20 In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein the second sequence is designed or modified in a manner that prevents its entry into the RNAi pathway as a guide sequence 25 or as a sequence that is complementary to a target nucleic acid (e.g., RNA) sequence. Such design or modifications are expected to enhance the activity of siNA and/or improve the specificity of siNA molecules of the invention. These modifications are also expected to minimize any off-target effects and/or associated toxicity.

30 In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary

to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence is incapable of acting as a guide sequence for mediating RNA interference.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence does not have a terminal 5'-hydroxyl (5'-OH) or 5'-phosphate group.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence comprises a terminal cap moiety at the 5'-end of said second sequence. In one embodiment, the terminal cap moiety comprises an inverted abasic, inverted deoxy abasic, inverted nucleotide moiety, a group shown in **Figure 10**, an alkyl or cycloalkyl group, a heterocycle, or any other group that prevents RNAi activity in which the second sequence serves as a guide sequence or template for RNAi.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence comprises a terminal cap moiety at the 5'-end and 3'-end of said second sequence. In one embodiment, each terminal cap moiety individually comprises an inverted abasic, inverted deoxy abasic, inverted nucleotide moiety, a group shown in **Figure 10**, an alkyl or cycloalkyl group, a heterocycle, or any other group that prevents RNAi activity in which the second sequence serves as a guide sequence or template for RNAi.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved specificity for down regulating or inhibiting the expression of a target nucleic acid (e.g., a DNA or RNA such as a gene or its corresponding RNA), comprising (a) introducing one or more chemical modifications

into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved specificity. In another embodiment, the chemical modification used to improve specificity comprises terminal cap modifications at the 5'-end, 3'-end, or both 5' and 3'-ends of the siNA molecule. The terminal cap modifications can comprise, for example, structures shown in **Figure 10** (e.g. inverted deoxyabasic moieties) or any other chemical modification that renders a portion of the siNA molecule (e.g. the sense strand) incapable of mediating RNA interference against an off target nucleic acid sequence. In a non-limiting example, a siNA molecule is designed such that only the antisense sequence of the siNA molecule can serve as a guide sequence for RISC mediated degradation of a corresponding target RNA sequence. This can be accomplished by rendering the sense sequence of the siNA inactive by introducing chemical modifications to the sense strand that preclude recognition of the sense strand as a guide sequence by RNAi machinery. In one embodiment, such chemical modifications comprise any chemical group at the 5'-end of the sense strand of the siNA, or any other group that serves to render the sense strand inactive as a guide sequence for mediating RNA interference. These modifications, for example, can result in a molecule where the 5'-end of the sense strand no longer has a free 5'-hydroxyl (5'-OH) or a free 5'-phosphate group (e.g., phosphate, diphosphate, triphosphate, cyclic phosphate etc.). Non-limiting examples of such siNA constructs are described herein, such as "Stab 9/10", "Stab 7/8", "Stab 7/19", "Stab 17/22", "Stab 23/24", "Stab 24/25", and "Stab 24/26" (e.g., any siNA having Stab 7, 9, 17, 23, or 24 sense strands) chemistries and variants thereof (see **Table IV**) wherein the 5'-end and 3'-end of the sense strand of the siNA do not comprise a hydroxyl group or phosphate group.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved specificity for down regulating or inhibiting the expression of a target nucleic acid (e.g., a DNA or RNA such as a gene or its corresponding RNA), comprising introducing one or more chemical modifications into the structure of a siNA molecule that prevent a strand or portion of the siNA molecule from acting as a template or guide sequence for RNAi activity. In one embodiment, the inactive strand or sense region of the siNA molecule is the sense strand or sense region of the siNA molecule, i.e. the strand or region of the siNA that does not have

complementarity to the target nucleic acid sequence. In one embodiment, such chemical modifications comprise any chemical group at the 5'-end of the sense strand or region of the siNA that does not comprise a 5'-hydroxyl (5'-OH) or 5'-phosphate group, or any other group that serves to render the sense strand or sense region inactive as a guide sequence for mediating RNA interference. Non-limiting examples of such siNA constructs are described herein, such as "Stab 9/10", "Stab 7/8", "Stab 7/19", "Stab 17/22", "Stab 23/24", "Stab 24/25", and "Stab 24/26" (e.g., any siNA having Stab 7, 9, 17, 23, or 24 sense strands) chemistries and variants thereof (see **Table IV**) wherein the 5'-end and 3'-end of the sense strand of the siNA do not comprise a hydroxyl group or phosphate group.

In one embodiment, the invention features a method for screening siNA molecules that are active in mediating RNA interference against a target nucleic acid sequence comprising (a) generating a plurality of unmodified siNA molecules, (b) screening the siNA molecules of step (a) under conditions suitable for isolating siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence, and (c) introducing chemical modifications (e.g. chemical modifications as described herein or as otherwise known in the art) into the active siNA molecules of (b). In one embodiment, the method further comprises re-screening the chemically modified siNA molecules of step (c) under conditions suitable for isolating chemically modified siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence.

In one embodiment, the invention features a method for screening chemically modified siNA molecules that are active in mediating RNA interference against a target nucleic acid sequence comprising (a) generating a plurality of chemically modified siNA molecules (e.g. siNA molecules as described herein or as otherwise known in the art), and (b) screening the siNA molecules of step (a) under conditions suitable for isolating chemically modified siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence.

The term "ligand" refers to any compound or molecule, such as a drug, peptide, 30 hormone, or neurotransmitter, that is capable of interacting with another compound, such as a receptor, either directly or indirectly. The receptor that interacts with a ligand can be

present on the surface of a cell or can alternately be an intercellular receptor. Interaction of the ligand with the receptor can result in a biochemical reaction, or can simply be a physical interaction or association.

5 In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing an excipient formulation to a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such excipients include polymers such as cyclodextrins, lipids, cationic lipids, polyamines, phospholipids, nanoparticles, receptors, ligands, and others.

10 10 In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing nucleotides having any of Formulae I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability.

15 15 In another embodiment, polyethylene glycol (PEG) can be covalently attached to siNA compounds of the present invention. The attached PEG can be any molecular weight, preferably from about 2,000 to about 50,000 daltons (Da).

20 The present invention can be used alone or as a component of a kit having at least one of the reagents necessary to carry out the *in vitro* or *in vivo* introduction of RNA to test samples and/or subjects. For example, preferred components of the kit include a siNA molecule of the invention and a vehicle that promotes introduction of the siNA into cells of interest as described herein (e.g., using lipids and other methods of transfection known in the art, see for example Beigelman *et al*, US 6,395,713). The kit can be used for target validation, such as in determining gene function and/or activity, or in drug 25 optimization, and in drug discovery (see for example Usman *et al.*, USSN 60/402,996). Such a kit can also include instructions to allow a user of the kit to practice the invention.

30 The term "short interfering nucleic acid", "siNA", "short interfering RNA", "siRNA", "short interfering nucleic acid molecule", "short interfering oligonucleotide molecule", or "chemically-modified short interfering nucleic acid molecule" as used herein refers to any nucleic acid molecule capable of inhibiting or down regulating gene

expression or viral replication, for example by mediating RNA interference "RNAi" or gene silencing in a sequence-specific manner; see for example Zamore *et al.*, 2000, *Cell*, 101, 25-33; Bass, 2001, *Nature*, 411, 428-429; Elbashir *et al.*, 2001, *Nature*, 411, 494-498; and Kreutzer *et al.*, International PCT Publication No. WO 00/44895; Zernicka-  
5 Goetz *et al.*, International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck *et al.*, International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.*, International PCT Publication No. WO 00/44914; Allshire, 2002, *Science*, 297, 1818-  
10 1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237; Hutvagner and Zamore, 2002, *Science*, 297, 2056-60; McManus *et al.*, 2002, *RNA*, 8, 842-850; Reinhart *et al.*, 2002, *Gene & Dev.*, 16, 1616-1626; and Reinhart & Bartel, 2002, *Science*, 297, 1831). Non limiting examples of siNA molecules of the invention are shown in **Figures 4-6**, and  
15 **Tables II and III** herein. For example the siNA can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e. each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for  
20 example wherein the double stranded region is about 15 to about 30, e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 base pairs; the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof (e.g.,  
25 about 15 to about 25 or more nucleotides of the siNA molecule are complementary to the target nucleic acid or a portion thereof). Alternatively, the siNA is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the  
30

siNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s). The siNA can be a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is 5 complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises 10 nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either *in vivo* or *in vitro* to generate an active siNA molecule capable of mediating RNAi. The siNA can also comprise a single 15 stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof (for example, where such siNA molecule does not require the presence within the siNA molecule of nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide can further comprise a terminal phosphate group, such 20 as a 5'-phosphate (see for example Martinez *et al.*, 2002, *Cell.*, 110, 563-574 and Schwarz *et al.*, 2002, *Molecular Cell*, 10, 537-568), or 5',3'-diphosphate. In certain embodiments, the siNA molecule of the invention comprises separate sense and antisense sequences or regions, wherein the sense and antisense regions are covalently 25 linked by nucleotide or non-nucleotide linkers molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der waals interactions, hydrophobic interactions, and/or stacking interactions. In certain embodiments, the siNA molecules of the invention comprise nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the siNA molecule of the invention interacts with nucleotide sequence of a target gene in a 30 manner that causes inhibition of expression of the target gene. As used herein, siNA molecules need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides. In certain

embodiments, the short interfering nucleic acid molecules of the invention lack 2'-hydroxy (2'-OH) containing nucleotides. Applicant describes in certain embodiments short interfering nucleic acids that do not require the presence of nucleotides having a 2'-hydroxy group for mediating RNAi and as such, short interfering nucleic acid molecules 5 of the invention optionally do not include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such siNA molecules that do not require the presence of ribonucleotides within the siNA molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. Optionally, siNA molecules can comprise 10 ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions. The modified short interfering nucleic acid molecules of the invention can also be referred to as short interfering modified oligonucleotides "siMON." As used herein, the term siNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA 15 (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically-modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA 20 interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics. For example, siNA molecules of the invention can be used to epigenetically silence genes at both the post-transcriptional level or the pre-transcriptional level. In a non-limiting example, epigenetic regulation of gene expression by siNA molecules of the invention can result from siNA mediated modification of chromatin structure or 25 methylation pattern to alter gene expression (see, for example, Verdel *et al.*, 2004, *Science*, 303, 672-676; Pal-Bhadra *et al.*, 2004, *Science*, 303, 669-672; Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237).

30 In one embodiment, a siNA molecule of the invention is a duplex forming oligonucleotide "DFO", (see for example **Figures 14-15** and Vaish *et al.*, USSN 10/727,780 filed December 3, 2003 and International PCT Application No. US04/16390, filed May 24, 2004).

In one embodiment, a siNA molecule of the invention is a multifunctional siNA, (see for example **Figures 16-21** and Jadhav *et al.*, USSN 60/543,480 filed February 10, 2004 and International PCT Application No. US04/16390, filed May 24, 2004). The multifunctional siNA of the invention can comprise sequence targeting, for example, two 5 regions of GPRA and/or AAA1 RNA (see for example target sequences in **Tables II and III**).

By “asymmetric hairpin” as used herein is meant a linear siNA molecule comprising an antisense region, a loop portion that can comprise nucleotides or non-nucleotides, and a sense region that comprises fewer nucleotides than the antisense 10 region to the extent that the sense region has enough complementary nucleotides to base pair with the antisense region and form a duplex with loop. For example, an asymmetric hairpin siNA molecule of the invention can comprise an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 15 to about 30, or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides) and a 15 loop region comprising about 4 to about 12 (e.g., about 4, 5, 6, 7, 8, 9, 10, 11, or 12) nucleotides, and a sense region having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides that are complementary to the antisense region. The asymmetric hairpin siNA molecule can also comprise a 5'-terminal phosphate group that can be chemically modified. The loop 20 portion of the asymmetric hairpin siNA molecule can comprise nucleotides, non-nucleotides, linker molecules, or conjugate molecules as described herein.

By “asymmetric duplex” as used herein is meant a siNA molecule having two separate strands comprising a sense region and an antisense region, wherein the sense region comprises fewer nucleotides than the antisense region to the extent that the sense 25 region has enough complementary nucleotides to base pair with the antisense region and form a duplex. For example, an asymmetric duplex siNA molecule of the invention can comprise an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 15 to about 30, or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides) and a sense region having about 3 to about 25 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides that are complementary to the antisense region.

By "modulate" is meant that the expression of the gene, or level of RNA molecule or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the 5 absence of the modulator. For example, the term "modulate" can mean "inhibit," but the use of the word "modulate" is not limited to this definition.

By "inhibit", "down-regulate", or "reduce", it is meant that the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, is 10 reduced below that observed in the absence of the nucleic acid molecules (e.g., siNA) of the invention. In one embodiment, inhibition, down-regulation or reduction with an siNA molecule is below that level observed in the presence of an inactive or attenuated molecule. In another embodiment, inhibition, down-regulation, or reduction with siNA molecules is below that level observed in the presence of, for example, an siNA molecule with scrambled sequence or with mismatches. In another embodiment, inhibition, down-regulation, or reduction of gene expression with a nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its 15 absence. In one embodiment, inhibition, down regulation, or reduction of gene expression is associated with post transcriptional silencing, such as RNAi mediated 20 cleavage of a target nucleic acid molecule (e.g. RNA) or inhibition of translation. In one embodiment, inhibition, down regulation, or reduction of gene expression is associated with pretranscriptional silencing.

By "gene", or "target gene", is meant a nucleic acid that encodes an RNA, for example, nucleic acid sequences including, but not limited to, structural genes encoding 25 a polypeptide. A gene or target gene can also encode a functional RNA (fRNA) or non-coding RNA (ncRNA), such as small temporal RNA (stRNA), micro RNA (miRNA), small nuclear RNA (snRNA), short interfering RNA (siRNA), small nucleolar RNA (snRNA), ribosomal RNA (rRNA), transfer RNA (tRNA) and precursor RNAs thereof. Such non-coding RNAs can serve as target nucleic acid molecules for siNA mediated 30 RNA interference in modulating the activity of fRNA or ncRNA involved in functional or regulatory cellular processes. Aberrant fRNA or ncRNA activity leading to disease

can therefore be modulated by siNA molecules of the invention. siNA molecules targeting fRNA and ncRNA can also be used to manipulate or alter the genotype or phenotype of a subject, organism or cell, by intervening in cellular processes such as genetic imprinting, transcription, translation, or nucleic acid processing (e.g., 5 transamination, methylation etc.). The target gene can be a gene derived from a cell, an endogenous gene, a transgene, or exogenous genes such as genes of a pathogen, for example a virus, which is present in the cell after infection thereof. The cell containing the target gene can be derived from or contained in any organism, for example a plant, animal, protozoan, virus, bacterium, or fungus. Non-limiting examples of plants include 10 monocots, dicots, or gymnosperms. Non-limiting examples of animals include vertebrates or invertebrates. Non-limiting examples of fungi include molds or yeasts. For a review, see for example Snyder and Gerstein, 2003, *Science*, 300, 258-260.

By "non-canonical base pair" is meant any non-Watson Crick base pair, such as mismatches and/or wobble base pairs, including flipped mismatches, single hydrogen 15 bond mismatches, trans-type mismatches, triple base interactions, and quadruple base interactions. Non-limiting examples of such non-canonical base pairs include, but are not limited to, AC reverse Hoogsteen, AC wobble, AU reverse Hoogsteen, GU wobble, AA N7 amino, CC 2-carbonyl-amino(H1)-N3-amino(H2), GA sheared, UC 4-carbonyl-amino, UU imino-carbonyl, AC reverse wobble, AU Hoogsteen, AU reverse Watson 20 Crick, CG reverse Watson Crick, GC N3-amino-amino N3, AA N1-amino symmetric, AA N7-amino symmetric, GA N7-N1 amino-carbonyl, GA+ carbonyl-amino N7-N1, GG N1-carbonyl symmetric, GG N3-amino symmetric, CC carbonyl-amino symmetric, CC N3-amino symmetric, UU 2-carbonyl-imino symmetric, UU 4-carbonyl-imino symmetric, AA amino-N3, AA N1-amino, AC amino 2-carbonyl, AC N3-amino, AC 25 N7-amino, AU amino-4-carbonyl, AU N1-imino, AU N3-imino, AU N7-imino, CC carbonyl-amino, GA amino-N1, GA amino-N7, GA carbonyl-amino, GA N3-amino, GC amino-N3, GC carbonyl-amino, GC N3-amino, GC N7-amino, GG amino-N7, GG carbonyl-imino, GG N7-amino, GU amino-2-carbonyl, GU carbonyl-imino, GU imino-2-carbonyl, GU N7-imino, psiU imino-2-carbonyl, UC 4-carbonyl-amino, UC imino-30 carbonyl, UU imino-4-carbonyl, AC C2-H-N3, GA carbonyl-C2-H, UU imino-4-carbonyl 2 carbonyl-C5-H, AC amino(A) N3(C)-carbonyl, GC imino amino-carbonyl, Gpsi imino-2-carbonyl amino-2- carbonyl, and GU imino amino-2-carbonyl base pairs.

By "G protein-coupled receptor for asthma susceptibility," "GPR154," or "GPRA" as used herein is meant, any G protein-coupled receptor protein, peptide, or polypeptide having any G protein-coupled receptor activity, such as encoded by GPRA Genbank Accession Nos. shown in **Table I**. The terms "G protein-coupled receptor for asthma susceptibility," "GPR154," or "GPRA" also refer to nucleic acid sequences encoding any G protein-coupled receptor protein, peptide, or polypeptide having G protein-coupled receptor activity. The terms "G protein-coupled receptor for asthma susceptibility," "GPR154," or "GPRA" are also meant to include other G protein-coupled receptor encoding sequence, such as other G protein-coupled receptor isoforms, mutant G protein-coupled receptor genes, splice variants of G protein-coupled receptor genes, and G protein-coupled receptor gene polymorphisms.

By "asthma-associated alternatively spliced gene 1" or "AAA1" as used herein is meant, any asthma-associated alternatively spliced protein, peptide, or polypeptide having any asthma-associated alternatively spliced gene activity, such as encoded by AAA1 Genbank Accession Nos. shown in **Table I**. The terms "asthma-associated alternatively spliced gene 1" or "AAA1" also refer to nucleic acid sequences encoding any asthma-associated alternatively spliced protein, peptide, or polypeptide having asthma-associated alternatively spliced gene activity. The terms "asthma-associated alternatively spliced gene 1" or "AAA1" are also meant to include other asthma-associated alternatively spliced gene encoding sequence, such as other asthma-associated alternatively spliced gene isoforms, mutant asthma-associated alternatively spliced gene genes, splice variants of asthma-associated alternatively spliced genes, and asthma-associated alternatively spliced gene polymorphisms.

By "homologous sequence" is meant, a nucleotide sequence that is shared by one or more polynucleotide sequences, such as genes, gene transcripts and/or non-coding polynucleotides. For example, a homologous sequence can be a nucleotide sequence that is shared by two or more genes encoding related but different proteins, such as different members of a gene family, different protein epitopes, different protein isoforms or completely divergent genes, such as a cytokine and its corresponding receptors. A homologous sequence can be a nucleotide sequence that is shared by two or more non-coding polynucleotides, such as noncoding DNA or RNA, regulatory sequences, introns,

and sites of transcriptional control or regulation. Homologous sequences can also include conserved sequence regions shared by more than one polynucleotide sequence. Homology does not need to be perfect homology (e.g., 100%), as partially homologous sequences are also contemplated by the instant invention (e.g., 99%, 98%, 97%, 96%, 5 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% etc.).

10 By "conserved sequence region" is meant, a nucleotide sequence of one or more regions in a polynucleotide does not vary significantly between generations or from one biological system, subject, or organism to another biological system, subject, or organism. The polynucleotide can include both coding and non-coding DNA and RNA.

15 By "sense region" is meant a nucleotide sequence of a siNA molecule having complementarity to an antisense region of the siNA molecule. In addition, the sense region of a siNA molecule can comprise a nucleic acid sequence having homology with a target nucleic acid sequence.

20 By "antisense region" is meant a nucleotide sequence of a siNA molecule having complementarity to a target nucleic acid sequence. In addition, the antisense region of a siNA molecule can optionally comprise a nucleic acid sequence having complementarity to a sense region of the siNA molecule.

25 By "target nucleic acid" is meant any nucleic acid sequence whose expression or activity is to be modulated. The target nucleic acid can be DNA or RNA.

30 By "complementarity" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity. Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner *et al.*, 1987, *CSH Symp. Quant. Biol.* LII pp.123-133; Frier *et al.*, 1986, *Proc. Nat. Acad. Sci. USA* 83:9373-9377; Turner *et al.*, 1987, *J. Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick

base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, or 10 nucleotides out of a total of 10 nucleotides in the first oligonucleotide being based paired to a second nucleic acid sequence having 10 nucleotides represents 50%, 60%, 70%, 80%, 90%, and 100% complementary respectively). "Perfectly complementary" means that all the 5 contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. In one embodiment, a siNA molecule of the invention comprises about 15 to about 30 or more (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more) nucleotides that are complementary to one or more target nucleic acid molecules or a portion thereof.

10 In one embodiment, siNA molecules of the invention that down regulate or reduce GPRA and/or AAA1 gene expression are used for preventing or treating inflammatory and/or respiratory diseases, disorders, and/or conditions in a subject or organism.

15 In one embodiment, the siNA molecules of the invention are used to treat inflammatory and/or respiratory diseases, disorders, and/or conditions in a subject or organism.

By "inflammatory disease" or "inflammatory condition" as used herein is meant any disease, condition, trait, genotype or phenotype characterized by an inflammatory or allergic process as is known in the art, such as inflammation, acute inflammation, chronic inflammation, respiratory disease, atherosclerosis, restenosis, asthma, allergic 20 rhinitis, atopic dermatitis, septic shock, rheumatoid arthritis, inflammatory bowel disease, inflammatory pelvic disease, pain, ocular inflammatory disease, celiac disease, Leigh Syndrome, Glycerol Kinase Deficiency, Familial eosinophilia (FE), autosomal recessive spastic ataxia, laryngeal inflammatory disease; Tuberculosis, Chronic cholecystitis, Bronchiectasis, Silicosis and other pneumoconioses, autoimmune disease, and any other 25 inflammatory disease, condition, trait, genotype or phenotype that can respond to the modulation of disease related gene expression in a cell or tissue, alone or in combination with other therapies.

By "autoimmune disease" or "autoimmune condition" as used herein is meant, any disease, condition, trait, genotype or phenotype characterized by autoimmunity as is 30 known in the art, such as multiple sclerosis, diabetes mellitus, lupus, celiac disease,

Crohn's disease, ulcerative colitis, Guillain-Barre syndrome, scleroderms, Goodpasture's syndrome, Wegener's granulomatosis, autoimmune epilepsy, Rasmussen's encephalitis, Primary biliary sclerosis, Sclerosing cholangitis, Autoimmune hepatitis, Addison's disease, Hashimoto's thyroiditis, Fibromyalgia, Menier's syndrome; transplantation  
5 rejection (e.g., prevention of allograft rejection) pernicious anemia, rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, Sjogren's syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, Reiter's syndrome, Grave's disease, and any other autoimmune disease, condition, trait, genotype or phenotype that can respond to the modulation of disease related gene expression in a cell or tissue, alone or  
10 in combination with other therapies.

By "respiratory disease" is meant, any disease or condition affecting the respiratory tract, such as asthma, chronic obstructive pulmonary disease or "COPD", allergic rhinitis, sinusitis, pulmonary vasoconstriction, inflammation, allergies, impeded respiration, respiratory distress syndrome, cystic fibrosis, pulmonary hypertension, 15 pulmonary vasoconstriction, emphysema, and any other respiratory disease, condition, trait, genotype or phenotype that can respond to the modulation of disease related gene expression in a cell or tissue, alone or in combination with other therapies..

In one embodiment of the present invention, each sequence of a siNA molecule of the invention is independently about 15 to about 30 nucleotides in length, in specific 20 embodiments about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. In another embodiment, the siNA duplexes of the invention independently comprise about 15 to about 30 base pairs (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30). In another embodiment, one or more strands of the siNA molecule of the invention independently comprises about 15 to about 30 25 nucleotides (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) that are complementary to a target nucleic acid molecule. In yet another embodiment, siNA molecules of the invention comprising hairpin or circular structures are about 35 to about 55 (e.g., about 35, 40, 45, 50 or 55) nucleotides in length, or about 38 to about 44 (e.g., about 38, 39, 40, 41, 42, 43, or 44) nucleotides in length and comprising about 15 30 to about 25 (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs.

Exemplary siNA molecules of the invention are shown in **Table II**. Exemplary synthetic siNA molecules of the invention are shown in **Table III** and/or **Figures 4-5**.

As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell can 5 be present in an organism, e.g., birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell). The cell can be of somatic or germ line origin, totipotent or pluripotent, dividing or non-dividing. The cell can also be derived from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell.

10 The siNA molecules of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through direct dermal application, transdermal application, or injection, with or without their incorporation in biopolymers. In 15 particular embodiments, the nucleic acid molecules of the invention comprise sequences shown in **Tables II-III** and/or **Figures 4-5**. Examples of such nucleic acid molecules consist essentially of sequences defined in these tables and figures. Furthermore, the chemically modified constructs described in **Table IV** can be applied to any siNA sequence of the invention.

20 In another aspect, the invention provides mammalian cells containing one or more siNA molecules of this invention. The one or more siNA molecules can independently be targeted to the same or different sites.

By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a  $\beta$ -D-25 ribofuranose moiety. The terms include double-stranded RNA, single-stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to 30 the end(s) of the siNA or internally, for example at one or more nucleotides of the RNA.

Nucleotides in the RNA molecules of the instant invention can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

5 By "subject" is meant an organism, which is a donor or recipient of explanted cells or the cells themselves. "Subject" also refers to an organism to which the nucleic acid molecules of the invention can be administered. A subject can be a mammal or mammalian cells, including a human or human cells.

10 The term "phosphorothioate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise a sulfur atom. Hence, the term phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

15 The term "phosphonoacetate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise an acetyl or protected acetyl group.

20 The term "thiophosphonoacetate" as used herein refers to an internucleotide linkage having Formula I, wherein Z comprises an acetyl or protected acetyl group and W comprises a sulfur atom or alternately W comprises an acetyl or protected acetyl group and Z comprises a sulfur atom.

25 The term "universal base" as used herein refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art (see for example Loakes, 2001, *Nucleic Acids Research*, 29, 2437-2447).

25. The term "acyclic nucleotide" as used herein refers to any nucleotide having an acyclic ribose sugar, for example where any of the ribose carbons (C1, C2, C3, C4, or C5), are independently or in combination absent from the nucleotide.

The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to for preventing or treating inflammatory and/or respiratory diseases, conditions, or disorders in a subject or organism.

5 For example, the siNA molecules can be administered to a subject or can be administered to other appropriate cells evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

10 In a further embodiment, the siNA molecules can be used in combination with other known treatments to prevent or treat inflammatory and/or respiratory diseases, conditions, or disorders in a subject or organism. For example, the described molecules could be used in combination with one or more known compounds, treatments, or procedures to prevent or treat inflammatory and/or respiratory diseases, conditions, or disorders in a subject or organism as are known in the art.

15 In one embodiment, the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention, in a manner which allows expression of the siNA molecule. For example, the vector can contain sequence(s) encoding both strands of a siNA molecule comprising a duplex. The vector can also contain sequence(s) encoding a single nucleic acid molecule that is self-complementary and thus forms a siNA molecule. Non-limiting examples of such 20 expression vectors are described in Paul *et al.*, 2002, *Nature Biotechnology*, 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology*, 19, 497; Lee *et al.*, 2002, *Nature Biotechnology*, 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725.

25 In another embodiment, the invention features a mammalian cell, for example, a human cell, including an expression vector of the invention.

In yet another embodiment, the expression vector of the invention comprises a sequence for a siNA molecule having complementarity to a RNA molecule referred to by a Genbank Accession numbers, for example Genbank Accession Nos. shown in Table I.

In one embodiment, an expression vector of the invention comprises a nucleic acid sequence encoding two or more siNA molecules, which can be the same or different.

In another aspect of the invention, siNA molecules that interact with target RNA molecules and down-regulate gene encoding target RNA molecules (for example target RNA molecules referred to by Genbank Accession numbers herein) are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecules bind and down-regulate gene function or expression via RNA interference (RNAi). Delivery of siNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1** shows a non-limiting example of a scheme for the synthesis of siNA molecules. The complementary siNA sequence strands, strand 1 and strand 2, are synthesized in tandem and are connected by a cleavable linkage, such as a nucleotide succinate or abasic succinate, which can be the same or different from the cleavable linker used for solid phase synthesis on a solid support. The synthesis can be either solid phase or solution phase, in the example shown, the synthesis is a solid phase synthesis. The synthesis is performed such that a protecting group, such as a dimethoxytrityl group, remains intact on the terminal nucleotide of the tandem oligonucleotide. Upon cleavage

and deprotection of the oligonucleotide, the two siNA strands spontaneously hybridize to form a siNA duplex, which allows the purification of the duplex by utilizing the properties of the terminal protecting group, for example by applying a trityl on purification method wherein only duplexes/oligonucleotides with the terminal protecting group are isolated.

Figure 2 shows a MALDI-TOF mass spectrum of a purified siNA duplex synthesized by a method of the invention. The two peaks shown correspond to the predicted mass of the separate siNA sequence strands. This result demonstrates that the siNA duplex generated from tandem synthesis can be purified as a single entity using a simple trityl-on purification methodology.

Figure 3 shows a non-limiting proposed mechanistic representation of target RNA degradation involved in RNAi. Double-stranded RNA (dsRNA), which is generated by RNA-dependent RNA polymerase (RdRP) from foreign single-stranded RNA, for example viral, transposon, or other exogenous RNA, activates the DICER enzyme that in turn generates siNA duplexes. Alternately, synthetic or expressed siNA can be introduced directly into a cell by appropriate means. An active siNA complex forms which recognizes a target RNA, resulting in degradation of the target RNA by the RISC endonuclease complex or in the synthesis of additional RNA by RNA-dependent RNA polymerase (RdRP), which can activate DICER and result in additional siNA molecules, thereby amplifying the RNAi response.

Figure 4A-F shows non-limiting examples of chemically-modified siNA constructs of the present invention. In the figure, N stands for any nucleotide (adenosine, guanosine, cytosine, uridine, or optionally thymidine, for example thymidine can be substituted in the overhanging regions designated by parenthesis (N N)). Various modifications are shown for the sense and antisense strands of the siNA constructs.

Figure 4A: The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all nucleotides present are ribonucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl

moiety wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all nucleotides present are ribonucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

**Figure 4B:** The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the sense and antisense strand.

**Figure 4C:** The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides,

which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the 5 antisense strand.

**Figure 4D:** The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, 10 deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, wherein all pyrimidine nucleotides that may be present are 2'- 15 deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown 20 as "s", optionally connects the (N N) nucleotides in the antisense strand.

**Figure 4E:** The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, 25 deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'- deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 30 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications

described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

**Figure 4F:** The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-deoxy nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand. The antisense strand of constructs A-F comprise sequence complementary to any target nucleic acid sequence of the invention. Furthermore, when a glyceryl moiety (L) is present at the 3'-end of the antisense strand for any construct shown in Figure 4 A-F, the modified internucleotide linkage is optional.

**Figure 5A-F** shows non-limiting examples of specific chemically-modified siNA sequences of the invention. A-F applies the chemical modifications described in **Figure 4A-F** to a GPRA siNA sequence. Such chemical modifications can be applied to any GPRA and/or AAA1 sequence and/or GPRA and/or AAA1 polymorphism sequence.

**Figure 6** shows non-limiting examples of different siNA constructs of the invention. The examples shown (constructs 1, 2, and 3) have 19 representative base pairs; however, different embodiments of the invention include any number of base pairs described herein. Bracketed regions represent nucleotide overhangs, for example,

comprising about 1, 2, 3, or 4 nucleotides in length, preferably about 2 nucleotides. Constructs 1 and 2 can be used independently for RNAi activity. Construct 2 can comprise a polynucleotide or non-nucleotide linker, which can optionally be designed as a biodegradable linker. In one embodiment, the loop structure shown in construct 2 can 5 comprise a biodegradable linker that results in the formation of construct 1 *in vivo* and/or *in vitro*. In another example, construct 3 can be used to generate construct 2 under the same principle wherein a linker is used to generate the active siNA construct 2 *in vivo* and/or *in vitro*, which can optionally utilize another biodegradable linker to generate the active siNA construct 1 *in vivo* and/or *in vitro*. As such, the stability and/or activity of 10 the siNA constructs can be modulated based on the design of the siNA construct for use *in vivo* or *in vitro* and/or *in vitro*.

**Figure 7A-C** is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate siNA hairpin constructs.

**Figure 7A:** A DNA oligomer is synthesized with a 5'-restriction site (R1) 15 sequence followed by a region having sequence identical (sense region of siNA) to a predetermined GPRA and/or AAA1 target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, which is followed by a loop sequence of defined sequence (X), comprising, for example, about 3 to about 10 nucleotides.

**Figure 7B:** The synthetic construct is then extended by DNA polymerase to 20 generate a hairpin structure having self-complementary sequence that will result in a siNA transcript having specificity for a GPRA and/or AAA1 target sequence and having self-complementary sense and antisense regions.

**Figure 7C:** The construct is heated (for example to about 95°C) to linearize the 25 sequence, thus allowing extension of a complementary second DNA strand using a primer to the 3'-restriction sequence of the first strand. The double-stranded DNA is then inserted into an appropriate vector for expression in cells. The construct can be designed such that a 3'-terminal nucleotide overhang results from the transcription, for example, by engineering restriction sites and/or utilizing a poly-U termination region as described 30 in Paul *et al.*, 2002, *Nature Biotechnology*, 29, 505-508.

**Figure 8A-C** is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate double-stranded siNA constructs.

5 **Figure 8A:** A DNA oligomer is synthesized with a 5'-restriction (R1) site sequence followed by a region having sequence identical (sense region of siNA) to a predetermined GPRA and/or AAA1 target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, and which is followed by a 3'-restriction site (R2) which is adjacent to a loop sequence of defined sequence (X).

10 **Figure 8B:** The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence.

15 **Figure 8C:** The construct is processed by restriction enzymes specific to R1 and R2 to generate a double-stranded DNA which is then inserted into an appropriate vector for expression in cells. The transcription cassette is designed such that a U6 promoter region flanks each side of the dsDNA which generates the separate sense and antisense strands of the siNA. Poly T termination sequences can be added to the constructs to generate U overhangs in the resulting transcript.

**Figure 9A-E** is a diagrammatic representation of a method used to determine target sites for siNA mediated RNAi within a particular target nucleic acid sequence, such as messenger RNA.

20 **Figure 9A:** A pool of siNA oligonucleotides are synthesized wherein the antisense region of the siNA constructs has complementarity to target sites across the target nucleic acid sequence, and wherein the sense region comprises sequence complementary to the antisense region of the siNA.

25 **Figure 9B&C:** (Figure 9B) The sequences are pooled and are inserted into vectors such that (Figure 9C) transfection of a vector into cells results in the expression of the siNA.

**Figure 9D:** Cells are sorted based on phenotypic change that is associated with modulation of the target nucleic acid sequence.

**Figure 9E:** The siNA is isolated from the sorted cells and is sequenced to identify efficacious target sites within the target nucleic acid sequence.

**Figure 10** shows non-limiting examples of different stabilization chemistries (1-10) that can be used, for example, to stabilize the 3'-end of siNA sequences of the invention, including (1) [3'-3']-inverted deoxyribose; (2) deoxyribonucleotide; (3) [5'-3']-3'-deoxyribonucleotide; (4) [5'-3']-ribonucleotide; (5) [5'-3']-3'-O-methyl ribonucleotide; (6) 3'-glyceryl; (7) [3'-5']-3'-deoxyribonucleotide; (8) [3'-3']-deoxyribonucleotide; (9) [5'-2']-deoxyribonucleotide; and (10) [5-3']-dideoxyribonucleotide. In addition to modified and unmodified backbone chemistries indicated in the figure, these chemistries can be combined with different backbone modifications as described herein, for example, backbone modifications having Formula I. In addition, the 2'-deoxy nucleotide shown 5' to the terminal modifications shown can be another modified or unmodified nucleotide or non-nucleotide described herein, for example modifications having any of Formulae I-VII or any combination thereof.

**Figure 11** shows a non-limiting example of a strategy used to identify chemically modified siNA constructs of the invention that are nuclease resistance while preserving the ability to mediate RNAi activity. Chemical modifications are introduced into the siNA construct based on educated design parameters (e.g. introducing 2'-mofications, base modifications, backbone modifications, terminal cap modifications etc). The modified construct in tested in an appropriate system (e.g. human serum for nuclease resistance, shown, or an animal model for PK/delivery parameters). In parallel, the siNA construct is tested for RNAi activity, for example in a cell culture system such as a luciferase reporter assay). Lead siNA constructs are then identified which possess a particular characteristic while maintaining RNAi activity, and can be further modified and assayed once again. This same approach can be used to identify siNA-conjugate molecules with improved pharmacokinetic profiles, delivery, and RNAi activity.

**Figure 12** shows non-limiting examples of phosphorylated siNA molecules of the invention, including linear and duplex constructs and asymmetric derivatives thereof.

**Figure 13** shows non-limiting examples of chemically modified terminal phosphate groups of the invention.

**Figure 14A** shows a non-limiting example of methodology used to design self complementary DFO constructs utilizing palindrome and/or repeat nucleic acid sequences that are identified in a target nucleic acid sequence. (i) A palindrome or repeat sequence is identified in a nucleic acid target sequence. (ii) A sequence is designed that is 5 complementary to the target nucleic acid sequence and the palindrome sequence. (iii) An inverse repeat sequence of the non-palindrome/repeat portion of the complementary sequence is appended to the 3'-end of the complementary sequence to generate a self complementary DFO molecule comprising sequence complementary to the nucleic acid target. (iv) The DFO molecule can self-assemble to form a double stranded 10 oligonucleotide. **Figure 14B** shows a non-limiting representative example of a duplex forming oligonucleotide sequence. **Figure 14C** shows a non-limiting example of the self assembly schematic of a representative duplex forming oligonucleotide sequence. **Figure 14D** shows a non-limiting example of the self assembly schematic of a representative duplex forming oligonucleotide sequence followed by interaction with a 15 target nucleic acid sequence resulting in modulation of gene expression.

**Figure 15** shows a non-limiting example of the design of self complementary DFO constructs utilizing palindrome and/or repeat nucleic acid sequences that are incorporated into the DFO constructs that have sequence complementary to any target nucleic acid sequence of interest. Incorporation of these palindrome/repeat sequences allow the 20 design of DFO constructs that form duplexes in which each strand is capable of mediating modulation of target gene expression, for example by RNAi. First, the target sequence is identified. A complementary sequence is then generated in which nucleotide or non-nucleotide modifications (shown as X or Y) are introduced into the complementary sequence that generate an artificial palindrome (shown as XYXYXY in 25 the Figure). An inverse repeat of the non-palindrome/repeat complementary sequence is appended to the 3'-end of the complementary sequence to generate a self complementary DFO comprising sequence complementary to the nucleic acid target. The DFO can self-assemble to form a double stranded oligonucleotide.

**Figure 16** shows non-limiting examples of multifunctional siNA molecules of the 30 invention comprising two separate polynucleotide sequences that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences. **Figure**

16A shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions 5 are situated at the 3'-ends of each polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. Figure 16B shows a non-limiting example of a multifunctional siNA molecule having a first 10 region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 5'-ends of each polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA 15 construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences.

Figure 17 shows non-limiting examples of multifunctional siNA molecules of the invention comprising a single polynucleotide sequence comprising distinct regions that are each capable of mediating RNAi directed cleavage of differing target nucleic acid 20 sequences. Figure 17A shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the second complementary region is situated at the 3'-end of the polynucleotide sequence in the 25 multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. Figure 17B shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid 30 sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first complementary region is situated at the 5'-end of the polynucleotide sequence in the

multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. In one embodiment, these multifunctional siNA constructs are processed in vivo or in vitro to generate multifunctional siNA constructs as shown in **Figure 16**.

Figure 18 shows non-limiting examples of multifunctional siNA molecules of the invention comprising two separate polynucleotide sequences that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences and wherein the multifunctional siNA construct further comprises a self complementary, palindrome, or repeat region, thus enabling shorter bifunctional siNA constructs that can mediate RNA interference against differing target nucleic acid sequences. **Figure 18A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 3'-ends of each polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 18B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 5'-ends of each polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences.

**Figure 19** shows non-limiting examples of multifunctional siNA molecules of the invention comprising a single polynucleotide sequence comprising distinct regions that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences and wherein the multifunctional siNA construct further comprises a self complementary, palindrome, or repeat region, thus enabling shorter bifunctional siNA constructs that can mediate RNA interference against differing target nucleic acid sequences. **Figure 19A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the second complementary region is situated at the 3'-end of the polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 19B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first complementary region is situated at the 5'-end of the polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. In one embodiment, these multifunctional siNA constructs are processed *in vivo* or *in vitro* to generate multifunctional siNA constructs as shown in **Figure 18**.

**Figure 20** shows a non-limiting example of how multifunctional siNA molecules of the invention can target two separate target nucleic acid molecules, such as separate RNA molecules encoding differing proteins, for example, a cytokine and its corresponding receptor, differing viral strains, a virus and a cellular protein involved in viral infection or replication, or differing proteins involved in a common or divergent

biologic pathway that is implicated in the maintenance of progression of disease. Each strand of the multifunctional siNA construct comprises a region having complementarity to separate target nucleic acid molecules. The multifunctional siNA molecule is designed such that each strand of the siNA can be utilized by the RISC complex to 5 initiate RNA interference mediated cleavage of its corresponding target. These design parameters can include destabilization of each end of the siNA construct (see for example Schwarz *et al.*, 2003, *Cell*, 115, 199-208). Such destabilization can be accomplished for example by using guanosine-cytidine base pairs, alternate base pairs (e.g., wobbles), or destabilizing chemically modified nucleotides at terminal nucleotide 10 positions as is known in the art.

Figure 21 shows a non-limiting example of how multifunctional siNA molecules of the invention can target two separate target nucleic acid sequences within the same target nucleic acid molecule, such as alternate coding regions of a RNA, coding and non-coding regions of a RNA, or alternate splice variant regions of a RNA. Each strand of 15 the multifunctional siNA construct comprises a region having complementarity to the separate regions of the target nucleic acid molecule. The multifunctional siNA molecule is designed such that each strand of the siNA can be utilized by the RISC complex to initiate RNA interference mediated cleavage of its corresponding target region. These design parameters can include destabilization of each end of the siNA construct (see for 20 example Schwarz *et al.*, 2003, *Cell*, 115, 199-208). Such destabilization can be accomplished for example by using guanosine-cytidine base pairs, alternate base pairs (e.g., wobbles), or destabilizing chemically modified nucleotides at terminal nucleotide positions as is known in the art.

## DETAILED DESCRIPTION OF THE INVENTION

### 25 Mechanism of Action of Nucleic Acid Molecules of the Invention

The discussion that follows discusses the proposed mechanism of RNA interference mediated by short interfering RNA as is presently known, and is not meant to be limiting and is not an admission of prior art. Applicant demonstrates herein that 30 chemically-modified short interfering nucleic acids possess similar or improved capacity to mediate RNAi as do siRNA molecules and are expected to possess improved stability

and activity *in vivo*; therefore, this discussion is not meant to be limiting only to siRNA and can be applied to siNA as a whole. By "improved capacity to mediate RNAi" or "improved RNAi activity" is meant to include RNAi activity measured *in vitro* and/or *in vivo* where the RNAi activity is a reflection of both the ability of the siNA to mediate RNAi and the stability of the siNAs of the invention. In this invention, the product of these activities can be increased *in vitro* and/or *in vivo* compared to an all RNA siRNA or a siNA containing a plurality of ribonucleotides. In some cases, the activity or stability of the siNA molecule can be decreased (i.e., less than ten-fold), but the overall activity of the siNA molecule is enhanced *in vitro* and/or *in vivo*.

RNA interference refers to the process of sequence specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al.*, 1998, *Nature*, 391, 806). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes which is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2', 5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as Dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from Dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes. Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal

RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence homologous to the siRNA. Cleavage of the target RNA takes place in the middle of the region complementary to the guide sequence of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). In addition, RNA interference can also involve small RNA (e.g., micro-RNA or miRNA) mediated gene silencing, presumably through cellular mechanisms that regulate chromatin structure and thereby prevent transcription of target gene sequences (see for example Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237). As such, siRNA molecules of the invention can be used to mediate gene silencing via interaction with RNA transcripts or alternately by interaction with particular gene sequences, wherein such interaction results in gene silencing either at the transcriptional level or post-transcriptional level.

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond *et al.*, 2000, *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature*, 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in *Drosophila* embryonic lysates has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21 nucleotide siRNA duplexes are most active when containing two 2-nucleotide 3'-terminal nucleotide overhangs. Furthermore, substitution of one or both siRNA strands with 2'-deoxy or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of 3'-terminal siRNA nucleotides with deoxy nucleotides was shown to be tolerated. Mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-

end (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell*, 107, 309); however, siRNA molecules lacking a 5'-phosphate are active when introduced exogenously, suggesting that 5'-phosphorylation of siRNA constructs may occur *in vivo*.

#### Synthesis of Nucleic Acid Molecules

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this 10 invention, small nucleic acid motifs ("small" refers to nucleic acid motifs no more than 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; *e.g.*, individual siNA oligonucleotide sequences or siNA sequences synthesized in tandem) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid 15 to invade targeted regions of protein and/or RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

Oligonucleotides (*e.g.*, certain modified oligonucleotides or portions of oligonucleotides lacking ribonucleotides) are synthesized using protocols known in the art, for example as described in Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-20 19, Thompson *et al.*, International PCT Publication No. WO 99/54459, Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, Brennan *et al.*, 1998, *Biotechnol Bioeng.*, 61, 33-45, and Brennan, U.S. Pat. No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of 25 oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 second coupling step for 2'-deoxy nucleotides or 2'-deoxy-2'-fluoro nucleotides. Table V outlines the amounts and the contact times of the 30 reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by

Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60  $\mu$ L of 0.11 M = 6.6  $\mu$ mol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60  $\mu$ L of 0.25 M = 15  $\mu$ mol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40  $\mu$ L of 0.11 M = 4.4  $\mu$ mol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40  $\mu$ L of 0.25 M = 10  $\mu$ mol) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% N-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM I<sub>2</sub>, 49 mM pyridine, 9% water in THF (PerSeptive Biosystems, Inc.). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

Deprotection of the DNA-based oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aqueous methylamine (1 mL) at 65 °C for 10 minutes. After cooling to -20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H<sub>2</sub>O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

The method of synthesis used for RNA including certain siNA molecules of the invention follows the procedure as described in Usman *et al.*, 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.*, 1990, *Nucleic Acids Res.*, 18, 5433; and Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684 Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting

example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2  $\mu$ mol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. Table V outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2  $\mu$ mol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60  $\mu$ L of 0.11 M = 6.6  $\mu$ mol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60  $\mu$ L of 0.25 M = 15  $\mu$ mol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120  $\mu$ L of 0.11 M = 13.2  $\mu$ mol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120  $\mu$ L of 0.25 M = 30  $\mu$ mol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM  $I_2$ , 49 mM pyridine, 9% water in THF (PerSeptive Biosystems, Inc.). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide 0.05 M in acetonitrile) is used.

Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to -20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H<sub>2</sub>O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous

TEA/HF/NMP solution (300  $\mu$ L of a solution of 1.5 mL N-methylpyrrolidinone, 750  $\mu$ L TEA and 1 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65 °C. After 1.5 h, the oligomer is quenched with 1.5 M  $\text{NH}_4\text{HCO}_3$ .

Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65 °C for 15 minutes. The vial is brought to room temperature TEA•3HF (0.1 mL) is added and the vial is heated at 65 °C for 15 minutes. The sample is cooled at -20 °C and then quenched with 1.5 M  $\text{NH}_4\text{HCO}_3$ .

For purification of the trityl-on oligomers, the quenched  $\text{NH}_4\text{HCO}_3$  solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 minutes. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

The average stepwise coupling yields are typically >98% (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including but not limited to 96-well format.

Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example, by ligation (Moore *et al.*, 1992, *Science* 256, 9923; Draper *et al.*, International PCT publication No. WO 93/23569; Shabarova *et al.*, 1991, *Nucleic Acids Research* 19, 4247; Bellon *et al.*, 1997, *Nucleosides & Nucleotides*, 16, 951; Bellon *et al.*, 1997, *Bioconjugate Chem.* 8, 204), or by hybridization following synthesis and/or deprotection.

The siNA molecules of the invention can also be synthesized via a tandem synthesis methodology as described in Example 1 herein, wherein both siNA strands are synthesized as a single contiguous oligonucleotide fragment or strand separated by a cleavable linker which is subsequently cleaved to provide separate siNA fragments or

strands that hybridize and permit purification of the siNA duplex. The linker can be a polynucleotide linker or a non-nucleotide linker. The tandem synthesis of siNA as described herein can be readily adapted to both multiwell/multiplate synthesis platforms such as 96 well or similarly larger multi-well platforms. The tandem synthesis of siNA as 5 described herein can also be readily adapted to large scale synthesis platforms employing batch reactors, synthesis columns and the like.

A siNA molecule can also be assembled from two distinct nucleic acid strands or fragments wherein one fragment includes the sense region and the second fragment includes the antisense region of the RNA molecule.

10 The nucleic acid molecules of the present invention can be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163). siNA constructs can be purified by gel electrophoresis using general methods or can be purified by high 15 pressure liquid chromatography (HPLC; see Wincott *et al.*, *supra*, the totality of which is hereby incorporated herein by reference) and re-suspended in water.

20 In another aspect of the invention, siNA molecules of the invention are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules.

Optimizing Activity of the nucleic acid molecule of the invention.

25 Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) can prevent their degradation by serum ribonucleases, which can increase their potency (see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 *Nature* 344, 565; Pieken *et al.*, 1991, *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman *et al.*, 30 International Publication No. WO 93/15187; and Rossi *et al.*, International Publication

No. WO 91/03162; Sproat, U.S. Pat. No. 5,334,711; Gold *et al.*, U.S. Pat. No. 6,300,074; and Burgin *et al.*, *supra*; all of which are incorporated by reference herein). All of the above references describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules described herein.

5 Modifications that enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant 10 enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-O-allyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TIBS*, 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin *et al.*, 1996, 15 *Biochemistry*, 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein *et al.*, *International Publication* PCT No. WO 92/07065; Perrault *et al.* *Nature*, 1990, 344, 565-568; Pieken *et al.* *Science*, 1991, 253, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.*, 1992, 17, 334-339; Usman *et al.* *International Publication* PCT No. WO 93/15187; Sproat, U.S. Pat. No. 20 5,334,711 and Beigelman *et al.*, 1995, *J. Biol. Chem.*, 270, 25702; Beigelman *et al.*, International PCT publication No. WO 97/26270; Beigelman *et al.*, U.S. Pat. No. 5,716,824; Usman *et al.*, U.S. Pat. No. 5,627,053; Woolf *et al.*, International PCT Publication No. WO 98/13526; Thompson *et al.*, USSN 60/082,404 which was filed on April 20, 1998; Karpeisky *et al.*, 1998, *Tetrahedron Lett.*, 39, 1131; Earnshaw and Gait, 25 1998, *Biopolymers (Nucleic Acid Sciences)*, 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.*, 67, 99-134; and Burlina *et al.*, 1997, *Bioorg. Med. Chem.*, 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such 30 publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into nucleic acid molecules without modulating catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify

the siNA nucleic acid molecules of the instant invention so long as the ability of siNA to promote RNAi in cells is not significantly inhibited.

While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorodithioate, and/or 5'-methylphosphonate linkages improves 5 stability, excessive modifications can cause some toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages should be minimized. The reduction in the concentration of these linkages should lower toxicity, resulting in increased efficacy and higher specificity of these molecules.

10 Short interfering nucleic acid (siNA) molecules having chemical modifications that maintain or enhance activity are provided. Such a nucleic acid is also generally more resistant to nucleases than an unmodified nucleic acid. Accordingly, the *in vitro* and/or *in vivo* activity should not be significantly lowered. In cases in which modulation is the goal, therapeutic nucleic acid molecules delivered exogenously should optimally be 15 stable within cells until translation of the target RNA has been modulated long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Improvements in the chemical synthesis of RNA and DNA (Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677; Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19 (incorporated by reference herein)) have expanded the 20 ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability, as described above.

In one embodiment, nucleic acid molecules of the invention include one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) G-clamp nucleotides. A G-clamp 25 nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.*, 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in 30 nucleic acid molecules of the invention results in both enhanced affinity and specificity to nucleic acid targets, complementary sequences, or template strands. In another

embodiment, nucleic acid molecules of the invention include one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) LNA "locked nucleic acid" nucleotides such as a 2', 4'-C methylene bicyclo nucleotide (see for example Wengel *et al.*, International PCT Publication No. WO 00/66604 and WO 99/14226).

5        In another embodiment, the invention features conjugates and/or complexes of siNA molecules of the invention. Such conjugates and/or complexes can be used to facilitate delivery of siNA molecules into a biological system, such as a cell. The conjugates and complexes provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes, altering the 10 pharmacokinetics, and/or modulating the localization of nucleic acid molecules of the invention. The present invention encompasses the design and synthesis of novel conjugates and complexes for the delivery of molecules, including, but not limited to, small molecules, lipids, cholesterol, phospholipids, nucleosides, nucleotides, nucleic acids, antibodies, toxins, negatively charged polymers and other polymers, for example 15 proteins, peptides, hormones, carbohydrates, polyethylene glycols, or polyamines, across cellular membranes. In general, the transporters described are designed to be used either individually or as part of a multi-component system, with or without degradable linkers. These compounds are expected to improve delivery and/or localization of nucleic acid molecules of the invention into a number of cell types originating from different tissues, 20 in the presence or absence of serum (see Sullenger and Cech, U.S. Pat. No. 5,854,038). Conjugates of the molecules described herein can be attached to biologically active molecules via linkers that are biodegradable, such as biodegradable nucleic acid linker molecules.

25        The term "biodegradable linker" as used herein, refers to a nucleic acid or non-nucleic acid linker molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule to a siNA molecule of the invention or the sense and antisense strands of a siNA molecule of the invention. The biodegradable linker is designed such that its stability can be modulated for a particular purpose, such as delivery to a particular tissue or cell type. The stability 30 of a nucleic acid-based biodegradable linker molecule can be modulated by using various chemistries, for example combinations of ribonucleotides, deoxyribonucleotides, and

chemically-modified nucleotides, such as 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O-allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker molecule can be a dimer, trimer, tetramer or longer nucleic acid molecule, for example, an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 5 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus-based linkage, for example, a phosphoramidate or phosphodiester linkage. The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

10 The term "biodegradable" as used herein, refers to degradation in a biological system, for example, enzymatic degradation or chemical degradation.

15 The term "biologically active molecule" as used herein refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system. Non-limiting examples of biologically active siNA molecules either alone or in combination with other molecules contemplated by the instant invention include 20 therapeutically active molecules such as antibodies, cholesterol, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siNA, dsRNA, allozymes, aptamers, decoys and analogs thereof. Biologically active molecules of the invention 25 also include molecules capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active molecules, for example, lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

25 The term "phospholipid" as used herein, refers to a hydrophobic molecule comprising at least one phosphorus group. For example, a phospholipid can comprise a phosphorus-containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

30 Therapeutic nucleic acid molecules (e.g., siNA molecules) delivered exogenously optimally are stable within cells until reverse transcription of the RNA has been modulated long enough to reduce the levels of the RNA transcript. The nucleic acid molecules are resistant to nucleases in order to function as effective intracellular

therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

5 In yet another embodiment, siNA molecules having chemical modifications that maintain or enhance enzymatic activity of proteins involved in RNAi are provided. Such nucleic acids are also generally more resistant to nucleases than unmodified nucleic acids. Thus, *in vitro* and/or *in vivo* the activity should not be significantly lowered.

10 Use of the nucleic acid-based molecules of the invention will lead to better treatments by affording the possibility of combination therapies (e.g., multiple siNA molecules targeted to different genes; nucleic acid molecules coupled with known small molecule modulators; or intermittent treatment with combinations of molecules, including different motifs and/or other chemical or biological molecules). The treatment of subjects with siNA molecules can also include combinations of different types of 15 nucleic acid molecules, such as enzymatic nucleic acid molecules (ribozymes), allozymes, antisense, 2,5-A oligoadenylate, decoys, and aptamers.

In another aspect a siNA molecule of the invention comprises one or more 5' and/or a 3'- cap structure, for example, on only the sense siNA strand, the antisense siNA strand, or both siNA strands.

20 By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Adamic *et al.*, U.S. Pat. No. 5,998,203, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'- 25 terminal (3'-cap) or may be present on both termini. In non-limiting examples, the 5'-cap includes, but is not limited to, glyceryl, inverted deoxy abasic residue (moiety); 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide; carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; *threo*-pentofuranosyl nucleotide; 30 acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-

dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging 5 methylphosphonate moiety. Non-limiting examples of cap moieties are shown in Figure 10.

Non-limiting examples of the 3'-cap include, but are not limited to, glyceryl, inverted deoxy abasic residue (moiety), 4', 5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl 10 phosphate; 1,3-diamino-2-propyl phosphate; 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide 15 moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

20 By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenine, guanine, cytosine, 25 uracil or thymine and therefore lacks a base at the 1'-position.

An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the 30 substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO<sub>2</sub> or N(CH<sub>3</sub>)<sub>2</sub>, amino, or SH. The term also includes alkenyl groups that are unsaturated hydrocarbon

groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably, it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the 5 substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO<sub>2</sub>, halogen, N(CH<sub>3</sub>)<sub>2</sub>, amino, or SH. The term "alkyl" also includes alkynyl groups that have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably, it is a lower alkynyl of from 1 to 7 carbons, 10 more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO<sub>2</sub> or N(CH<sub>3</sub>)<sub>2</sub>, amino or SH.

Such alkyl groups can also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group that has at 15 least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described 20 above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl 25 pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

By "nucleotide" as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at 30 the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the

sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see, for example, Usman and McSwiggen, *supra*; Eckstein *et al.*, International PCT Publication No. WO 92/07065; Usman *et al.*, International PCT Publication No. 5 WO 93/15187; Uhlman & Peyman, *supra*, all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al.*, 1994, *Nucleic Acids Res.* 22, 2183. Some of the non-limiting examples of base modifications that can be introduced into nucleic acid molecules include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 10 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (e.g., 5-methylcytidine), 5-alkyluridines (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (e.g. 6-methyluridine), propyne, and others (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090; 15 Uhlman & Peyman, *supra*). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents.

In one embodiment, the invention features modified siNA molecules, with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, phosphotriester, morpholino, amide carbamate, carboxymethyl, acetamide, polyamide, sulfonate, sulfonamide, sulfamate, 20 formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications, see Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties*, in *Modern Synthetic Methods*, VCH, 331-417, and Mesmaeker *et al.*, 1994, *Novel Backbone Replacements for Oligonucleotides*, in *Carbohydrate Modifications in Antisense Research*, ACS, 24-39.

25 By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, see for example Adamic *et al.*, U.S. Pat. No. 5,998,203.

By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, or uracil joined to the 1' carbon of  $\beta$ -D-ribo-furanose.

By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate. Non-limiting examples of modified nucleotides are shown by Formulae I-VII and/or other modifications described herein.

5        In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH<sub>2</sub> or 2'-O- NH<sub>2</sub>, which can be modified or unmodified. Such modified groups are described, for example, in Eckstein *et al.*, U.S. Pat. No. 5,672,695 and Matulic-Adamic *et al.*, U.S. Pat. No. 6,248,878, which are both incorporated by reference in their entireties.

10      Various modifications to nucleic acid siNA structure can be made to enhance the utility of these molecules. Such modifications will enhance shelf-life, half-life *in vitro*, stability, and ease of introduction of such oligonucleotides to the target site, *e.g.*, to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

15      Administration of Nucleic Acid Molecules

A siNA molecule of the invention can be adapted for use to prevent or treat inflammatory and/or respiratory diseases, conditions, or disorders, and/or any other trait, disease, disorder or condition that is related to or will respond to the levels of GPRA and/or AAA1 in a cell or tissue, alone or in combination with other therapies. For example, a siNA molecule can comprise a delivery vehicle, including liposomes, for administration to a subject, carriers and diluents and their salts, and/or can be present in pharmaceutically acceptable formulations. Methods for the delivery of nucleic acid molecules are described in Akhtar *et al.*, 1992, *Trends Cell Bio.*, 2, 139; *Delivery Strategies for Antisense Oligonucleotide Therapeutics*, ed. Akhtar, 1995, Maurer *et al.*, 1999, *Mol. Membr. Biol.*, 16, 129-140; Hofland and Huang, 1999, *Handb. Exp. Pharmacol.*, 137, 165-192; and Lee *et al.*, 2000, *ACS Symp. Ser.*, 752, 184-192, all of which are incorporated herein by reference. Beigelman *et al.*, U.S. Pat. No. 6,395,713 and Sullivan *et al.*, PCT WO 94/02595 further describe the general methods for delivery of nucleic acid molecules. These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells by a

variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as biodegradable polymers, hydrogels, cyclodextrins (see for example Gonzalez *et al.*, 1999, *Bioconjugate Chem.*, 10, 1068-1074; Wang *et al.*, International PCT 5 publication Nos. WO 03/47518 and WO 03/46185), poly(lactic-co-glycolic)acid (PLGA) and PLCA microspheres (see for example US Patent 6,447,796 and US Patent Application Publication No. US 2002130430), biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO 00/53722). Alternatively, the nucleic 10 acid/vehicle combination is locally delivered by direct injection or by use of an infusion pump. Direct injection of the nucleic acid molecules of the invention, whether subcutaneous, intramuscular, or intradermal, can take place using standard needle and syringe methodologies, or by needle-free technologies such as those described in Conry 15 *et al.*, 1999, *Clin. Cancer Res.*, 5, 2330-2337 and Barry *et al.*, International PCT Publication No. WO 99/31262. The molecules of the instant invention can be used as pharmaceutical agents. Pharmaceutical agents prevent, modulate the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a subject.

In another embodiment, the nucleic acid molecules of the invention can also be 20 formulated or complexed with polyethyleneimine and derivatives thereof, such as polyethyleneimine-polyethyleneglycol-N-acetylgalactosamine (PEI-PEG-GAL) or polyethyleneimine-polyethyleneglycol-tri-N-acetylgalactosamine (PEI-PEG-triGAL) derivatives. In one embodiment, the nucleic acid molecules of the invention are formulated as described in United States Patent Application Publication No. 25 20030077829, incorporated by reference herein in its entirety.

In one embodiment, a siNA molecule of the invention is complexed with membrane disruptive agents such as those described in U.S. Patent Application Publication No. 20010007666, incorporated by reference herein in its entirety including the drawings. In another embodiment, the membrane disruptive agent or agents and the 30 siNA molecule are also complexed with a cationic lipid or helper lipid molecule, such as

those lipids described in U.S. Patent No. 6,235,310, incorporated by reference herein in its entirety including the drawings.

In one embodiment, a siNA molecule of the invention is complexed with delivery systems as described in U.S. Patent Application Publication No. 2003077829 and 5 International PCT Publication Nos. WO 00/03683 and WO 02/087541, all incorporated by reference herein in their entirety including the drawings.

In one embodiment, the nucleic acid molecules of the invention are administered via pulmonary delivery, such as by inhalation of an aerosol or spray dried formulation administered by an inhalation device or nebulizer, providing rapid local uptake of the 10 nucleic acid molecules into relevant pulmonary tissues. Solid particulate compositions containing respirable dry particles of micronized nucleic acid compositions can be prepared by grinding dried or lyophilized nucleic acid compositions, and then passing the micronized composition through, for example, a 400 mesh screen to break up or separate out large agglomerates. A solid particulate composition comprising the nucleic acid 15 compositions of the invention can optionally contain a dispersant which serves to facilitate the formation of an aerosol as well as other therapeutic compounds. A suitable dispersant is lactose, which can be blended with the nucleic acid compound in any suitable ratio, such as a 1 to 1 ratio by weight.

Aerosols of liquid particles comprising a nucleic acid composition of the invention 20 can be produced by any suitable means, such as with a nebulizer (see for example US 4,501,729). Nebulizers are commercially available devices which transform solutions or suspensions of an active ingredient into a therapeutic aerosol mist either by means of acceleration of a compressed gas, typically air or oxygen, through a narrow venturi orifice or by means of ultrasonic agitation. Suitable formulations for use in nebulizers 25 comprise the active ingredient in a liquid carrier in an amount of up to 40% w/w preferably less than 20% w/w of the formulation. The carrier is typically water or a dilute aqueous alcoholic solution, preferably made isotonic with body fluids by the addition of, for example, sodium chloride or other suitable salts. Optional additives include preservatives if the formulation is not prepared sterile, for example, methyl 30 hydroxybenzoate, anti-oxidants, flavorings, volatile oils, buffering agents and emulsifiers and other formulation surfactants. The aerosols of solid particles comprising the active

composition and surfactant can likewise be produced with any solid particulate aerosol generator. Aerosol generators for administering solid particulate therapeutics to a subject produce particles which are respirable, as explained above, and generate a volume of aerosol containing a predetermined metered dose of a therapeutic composition at a rate 5 suitable for human administration. One illustrative type of solid particulate aerosol generator is an insufflator. Suitable formulations for administration by insufflation include finely comminuted powders which can be delivered by means of an insufflator. In the insufflator, the powder, e.g., a metered dose thereof effective to carry out the treatments described herein, is contained in capsules or cartridges, typically made of 10 gelatin or plastic, which are either pierced or opened in situ and the powder delivered by air drawn through the device upon inhalation or by means of a manually-operated pump. The powder employed in the insufflator consists either solely of the active ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent, such as lactose, and an optional surfactant. The active ingredient typically comprises from 0.1 to 15 100 w/w of the formulation. A second type of illustrative aerosol generator comprises a metered dose inhaler. Metered dose inhalers are pressurized aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in a liquified propellant. During use these devices discharge the formulation through a valve adapted to deliver a metered volume to produce a fine particle spray containing the active 20 ingredient. Suitable propellants include certain chlorofluorocarbon compounds, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane and mixtures thereof. The formulation can additionally contain one or more co-solvents, for example, ethanol, emulsifiers and other formulation surfactants, such as oleic acid or sorbitan trioleate, anti-oxidants and suitable flavoring agents. Other methods for 25 pulmonary delivery are described in, for example US Patent Application No. 20040037780, and US Patent Nos. 6,592,904; 6,582,728; 6,565,885.

In one embodiment, delivery systems of the invention include, for example, aqueous and nonaqueous gels, creams, multiple emulsions, microemulsions, liposomes, ointments, aqueous and nonaqueous solutions, lotions, aerosols, hydrocarbon bases and 30 powders, and can contain excipients such as solubilizers, permeation enhancers (e.g., fatty acids, fatty acid esters, fatty alcohols and amino acids), and hydrophilic polymers (e.g., polycarbophil and polyvinylpyrrolidone). In one embodiment, the pharmaceutically

acceptable carrier is a liposome or a transdermal enhancer. Examples of liposomes which can be used in this invention include the following: (1) CellFectin, 1:1.5 (M/M) liposome formulation of the cationic lipid N,N,N,N-tetramethyl-N,N,N,N-tetrapalmitoyl-spermine and dioleoyl phosphatidylethanolamine (DOPE) (GIBCO BRL); (2) Cytofectin 5 GSV, 2:1 (M/M) liposome formulation of a cationic lipid and DOPE (Glen Research); (3) DOTAP (N-[1-(2,3-dioleyloxy)-N,N,N,N-tetramethylammoniummethylsulfate] (Boehringer Manheim); and (4) Lipofectamine, 3:1 (M/M) liposome formulation of the polycationic lipid DOSPA and the neutral lipid DOPE (GIBCO BRL).

In one embodiment, delivery systems of the invention include patches, tablets, 10 suppositories, pessaries, gels and creams, and can contain excipients such as solubilizers and enhancers (e.g., propylene glycol, bile salts and amino acids), and other vehicles (e.g., polyethylene glycol, fatty acid esters and derivatives, and hydrophilic polymers such as hydroxypropylmethylcellulose and hyaluronic acid).

In one embodiment, siNA molecules of the invention are formulated or complexed 15 with polyethylenimine (e.g., linear or branched PEI) and/or polyethylenimine derivatives, including for example grafted PEIs such as galactose PEI, cholesterol PEI, antibody derivatized PEI, and polyethylene glycol PEI (PEG-PEI) derivatives thereof (see for example Ogris *et al.*, 2001, *AAPA PharmSci*, 3, 1-11; Furgeson *et al.*, 2003, *Bioconjugate Chem.*, 14, 840-847; Kunath *et al.*, 2002, *Pharmaceutical Research*, 19, 20 810-817; Choi *et al.*, 2001, *Bull. Korean Chem. Soc.*, 22, 46-52; Bettinger *et al.*, 1999, *Bioconjugate Chem.*, 10, 558-561; Peterson *et al.*, 2002, *Bioconjugate Chem.*, 13, 845-854; Erbacher *et al.*, 1999, *Journal of Gene Medicine Preprint*, 1, 1-18; Godbey *et al.*, 1999, *PNAS USA*, 96, 5177-5181; Godbey *et al.*, 1999, *Journal of Controlled Release*, 60, 149-160; Diebold *et al.*, 1999, *Journal of Biological Chemistry*, 274, 19087-19094; 25 Thomas and Klibanov, 2002, *PNAS USA*, 99, 14640-14645; and Sagara, US 6,586,524, incorporated by reference herein.

In one embodiment, a siNA molecule of the invention comprises a bioconjugate, for example a nucleic acid conjugate as described in Vargeese *et al.*, USSN 10/427,160, filed April 30, 2003; US 6,528,631; US 6,335,434; US 6,235,886; US 6,153,737; US 30 5,214,136; US 5,138,045, all incorporated by reference herein.

Thus, the invention features a pharmaceutical composition comprising one or more nucleic acid(s) of the invention in an acceptable carrier, such as a stabilizer, buffer, and the like. The polynucleotides of the invention can be administered (e.g., RNA, DNA or protein) and introduced to a subject by any standard means, with or without stabilizers, 5 buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention can also be formulated and used as creams, gels, sprays, oils and other suitable compositions for topical, dermal, or transdermal administration as is known in the art.

10 The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, e.g., acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

15 A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, e.g., systemic or local administration, into a cell or subject, including for example a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (i.e., a cell to which the negatively charged nucleic acid is desirable for delivery). For 20 example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

25 In one embodiment, siNA molecules of the invention are administered to a subject by systemic administration in a pharmaceutically acceptable composition or formulation. By "systemic administration" is meant *in vivo* systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes that lead to systemic absorption include, without limitation: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes exposes the siNA molecules of the 30 invention to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a

liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation that can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is 5 also useful. This approach can provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells.

By "pharmaceutically acceptable formulation" or "pharmaceutically acceptable composition" is meant, a composition or formulation that allows for the effective 10 distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85);, biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery (Emerich, DF *et al.*, 15 1999, *Cell Transplant*, 8, 47-58); and loaded nanoparticles, such as those made of polybutylcyanoacrylate. Other non-limiting examples of delivery strategies for the nucleic acid molecules of the instant invention include material described in Boado *et al.*, 1998, *J. Pharm. Sci.*, 87, 1308-1315; Tyler *et al.*, 1999, *FEBS Lett.*, 421, 280-284; Pardridge *et al.*, 1995, *PNAS USA*, 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery 20 Rev.*, 15, 73-107; Aldrian-Herrada *et al.*, 1998, *Nucleic Acids Res.*, 26, 4910-4916; and Tyler *et al.*, 1999, *PNAS USA*, 96, 7053-7058.

The invention also features the use of the composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer a method for 25 increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic *et al. Chem. Rev.* 1995, 95, 2601-2627; Ishiwata *et al., Chem. Pharm. Bull.* 1995, 43, 1005-1011). Such liposomes have been shown to accumulate 30 selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al., Science* 1995, 267, 1275-1276; Oku *et al.*, 1995, *Biochim.*

*Biophys. Acta*, 1238, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al.*, *J. Biol. Chem.* 1995, 42, 24864-24870; Choi *et al.*, International PCT Publication No. WO 96/10391; Ansell *et al.*, International PCT Publication No. WO 96/10390; Holland *et al.*, International PCT Publication No. WO 96/10392). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

10 The present invention also includes compositions prepared for storage or administration that include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. 15 Gennaro edit. 1985), hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

20 A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors that those skilled in the medical arts will recognize. 25 Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and/or vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or

intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

10 Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents; such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia; and lubricating agents, for example magnesium 15 stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed.

20 Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

25 Aqueous suspensions contain the active materials in a mixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-

methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain 5 aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene 10 sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral 15 oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid

Dispersible powders and granules suitable for preparation of an aqueous 20 suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

25 Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for 30 example sorbitan monooleate, and condensation products of the said partial esters with

ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a 5 demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in 10 a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, 15 fatty acids such as oleic acid find use in the preparation of injectables.

The nucleic acid molecules of the invention can also be administered in the form of suppositories, e.g., for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the 20 rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local 25 anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per subject per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending

upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

It is understood that the specific dose level for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, 5 body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and 10 drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The nucleic acid molecules of the present invention can also be administered to a subject in combination with other therapeutic compounds to increase the overall 15 therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

In one embodiment, the invention comprises compositions suitable for administering nucleic acid molecules of the invention to specific cell types. For example, the asialoglycoprotein receptor (ASGPr) (Wu and Wu, 1987, *J. Biol. Chem.* 262, 4429-4432) is unique to hepatocytes and binds branched galactose-terminal 20 glycoproteins, such as asialoorosomucoid (ASOR). In another example, the folate receptor is overexpressed in many cancer cells. Binding of such glycoproteins, synthetic glycoconjugates, or folates to the receptor takes place with an affinity that strongly depends on the degree of branching of the oligosaccharide chain, for example, 25 triantennary structures are bound with greater affinity than biantennary or monoantennary chains (Baenziger and Fiete, 1980, *Cell*, 22, 611-620; Connolly *et al.*, 1982, *J. Biol. Chem.*, 257, 939-945). Lee and Lee, 1987, *Glycoconjugate J.*, 4, 317-328, obtained this high specificity through the use of N-acetyl-D-galactosamine as the carbohydrate moiety, 30 which has higher affinity for the receptor, compared to galactose. This "clustering effect" has also been described for the binding and uptake of mannosyl-terminating

glycoproteins or glycoconjugates (Ponpipom *et al.*, 1981, *J. Med. Chem.*, 24, 1388-1395). The use of galactose, galactosamine, or folate based conjugates to transport exogenous compounds across cell membranes can provide a targeted delivery approach to, for example, the treatment of liver disease, cancers of the liver, or other cancers. The 5 use of bioconjugates can also provide a reduction in the required dose of therapeutic compounds required for treatment. Furthermore, therapeutic bioavailability, pharmacodynamics, and pharmacokinetic parameters can be modulated through the use of nucleic acid bioconjugates of the invention. Non-limiting examples of such bioconjugates are described in Vargeese *et al.*, USSN 10/201,394, filed August 13, 2001; 10 and Matulic-Adamic *et al.*, USSN 60/362,016, filed March 6, 2002.

Alternatively, certain siNA molecules of the instant invention can be expressed within cells from eukaryotic promoters (e.g., Izant and Weintraub, 1985, *Science*, 229, 345; McGarry and Lindquist, 1986, *Proc. Natl. Acad. Sci., USA* 83, 399; Scanlon *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88, 10591-5; Kashani-Sabet *et al.*, 1992, *Antisense 15 Res. Dev.*, 2, 3-15; Dropulic *et al.*, 1992, *J. Virol.*, 66, 1432-41; Weerasinghe *et al.*, 1991, *J. Virol.*, 65, 5531-4; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Sarver *et al.*, 1990 *Science*, 247, 1222-1225; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Good *et al.*, 1997, *Gene Therapy*, 4, 45. Those skilled in the art realize that any nucleic acid can be 20 expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a enzymatic nucleic acid (Draper *et al.*, PCT WO 93/23569, and Sullivan *et al.*, PCT WO 94/02595; Ohkawa *et al.*, 1992, *Nucleic Acids Symp. Ser.*, 27, 15-6; Taira *et al.*, 1991, *Nucleic Acids Res.*, 19, 5125-30; Ventura *et al.*, 1993, *Nucleic Acids Res.*, 21, 3249-55; 25 Chowrira *et al.*, 1994, *J. Biol. Chem.*, 269, 25856.

In another aspect of the invention, RNA molecules of the present invention can be expressed from transcription units (see for example Couture *et al.*, 1996, *TIG.*, 12, 510) inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited 30 to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. In another embodiment, pol III based constructs are used to express nucleic acid molecules of the invention (see

for example Thompson, U.S. Pats. Nos. 5,902,880 and 6,146,886). The recombinant vectors capable of expressing the siNA molecules can be delivered as described above, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly 5 administered as necessary. Once expressed, the siNA molecule interacts with the target mRNA and generates an RNAi response. Delivery of siNA molecule expressing vectors can be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired 10 target cell (for a review see Couture *et al.*, 1996, *TIG.*, 12, 510).

In one aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the instant invention. The expression vector can encode one or both strands of a siNA duplex, or a single self-complementary strand that self hybridizes into a siNA duplex. The nucleic acid sequences encoding the 15 siNA molecules of the instant invention can be operably linked in a manner that allows expression of the siNA molecule (see for example Paul *et al.*, 2002, *Nature Biotechnology*, 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology*, 19, 497; Lee *et al.*, 2002, *Nature Biotechnology*, 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725).

20 In another aspect, the invention features an expression vector comprising: a) a transcription initiation region (e.g., eukaryotic pol I, II or III initiation region); b) a transcription termination region (e.g., eukaryotic pol I, II or III termination region); and c) a nucleic acid sequence encoding at least one of the siNA molecules of the instant invention, wherein said sequence is operably linked to said initiation region and said 25 termination region in a manner that allows expression and/or delivery of the siNA molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the siNA of the invention; and/or an intron (intervening sequences).

Transcription of the siNA molecule sequences can be driven from a promoter for 30 eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters are expressed at high levels in

all cells; the levels of a given pol II promoter in a given cell type depends on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, 5 *Proc. Natl. Acad. Sci. U S A*, 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res.*, 21, 2867-72; Lieber *et al.*, 1993, *Methods Enzymol.*, 217, 47-66; Zhou *et al.*, 1990, *Mol. Cell. Biol.*, 10, 4529-37). Several investigators have demonstrated that nucleic acid molecules expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Ojwang *et al.*, 1992, *Proc. 10 Natl. Acad. Sci. U S A*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Yu *et al.*, 1993, *Proc. Natl. Acad. Sci. U S A*, 90, 6340-4; L'Huillier *et al.*, 1992, *EMBO J.*, 11, 4411-8; Lisziewicz *et al.*, 1993, *Proc. Natl. Acad. Sci. U. S. A.*, 90, 8000-4; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Sullenger & Cech, 1993, *Science*, 262, 1566). More specifically, transcription units such as the ones derived from genes 15 encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as siNA in cells (Thompson *et al.*, *supra*; Couture and Stinchcomb, 1996, *supra*; Noonberg *et al.*, 1994, *Nucleic Acid Res.*, 22, 2830; Noonberg *et al.*, U.S. Pat. No. 5,624,803; Good *et al.*, 1997, *Gene Ther.*, 4, 45; Beigelman *et al.*, International PCT Publication No. WO 96/18736. 20 The above siNA transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, 1996, *supra*).  
25 In another aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the siNA molecules of the invention in a manner that allows expression of that siNA molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; and c) a nucleic acid sequence encoding at least one strand of the siNA molecule, 30 wherein the sequence is operably linked to the initiation region and the termination region in a manner that allows expression and/or delivery of the siNA molecule.

In another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; and d) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the open reading frame and the termination region in a manner that allows expression and/or delivery of the siNA molecule. In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; and d) a nucleic acid sequence encoding at least one siNA molecule, wherein the sequence is operably linked to the initiation region, the intron and the termination region in a manner which allows expression and/or delivery of the nucleic acid molecule.

In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; and e) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the intron, the open reading frame and the termination region in a manner which allows expression and/or delivery of the siNA molecule.

#### GPRA and AAA1 Biology and Biochemistry

The following discussion is adapted from the OMIM database entry for g protein-coupled receptor for asthma susceptibility; GPRA, vasopressin receptor-related receptor 1; vrr1, pgr14 and asthma-associated alternatively spliced gene 1

To positionally clone genes conferring susceptibility to asthma and showing linkage to chromosome 7p, Laitinen *et al.*, 2004, *Science*, 304, 300-304, used a hierarchical genotyping approach to identify a 133-kb risk-conferring segment of chromosome 7p. The segment was examined for specific genes resulting in the identification of a gene designated GPRA for G protein-coupled receptor for asthma susceptibility. The 133-kb segment spans from intron 2 to intron 5 of GPRA. Northern blot hybridization with a 1,285-bp full-length GPRA cDNA probe identified a 2.4-kb transcript in all tissues examined. GPRA expression was much higher in the ciliated

cells of the respiratory epithelium from asthma patients compared with those from normal control patients. Asthmatic smooth muscle immunohistochemically stained strongly positive for GPRA isoform B, in contrast to the negative finding in controls. In addition, a higher level of GPRA expression was also found in mRNA from lungs of 5 sensitized versus control mice after inhaled ovalbumin challenge.

Another risk conferring segment identified by Laitinen *et al.*, *supra*, referred to as AAA1 for asthma-associated alternatively spliced gene 1, lies on the opposite DNA strand from GPRA and showed only weak homologies to known proteins. AAA1 exhibits complex alternative splicing. Laitinen *et al.*, *supra* concluded that several lines 10 of evidence suggested that AAA1 may not represent a protein-coding gene, although its expression was modified by the haplotype. The longest open-reading frame comprised only 74 potential amino acids, and *in vitro* translation failed to yield a stable polypeptide. Transiently transfected cells did not produce recombinant protein. Polyclonal peptide 15 antibodies detected the antigen but no proteins in Western blots or immunohistochemistry.

The use of small interfering nucleic acid molecules targeting GPRA and AAA1, such as disease related alleles of GPRA and/or AAA1, therefore provides a class of novel therapeutic agents that can be used in the the treatment of asthma and associated conditions that can respond to modulation of GPRA and/or AAA1 levels in a cell, tissue, 20 or subject.

Examples:

The following are non-limiting examples showing the selection, isolation, synthesis and activity of nucleic acids of the instant invention.

Example 1: Tandem synthesis of siNA constructs

25 Exemplary siNA molecules of the invention are synthesized in tandem using a cleavable linker, for example, a succinyl-based linker. Tandem synthesis as described herein is followed by a one-step purification process that provides RNAi molecules in high yield. This approach is highly amenable to siNA synthesis in support of high

throughput RNAi screening, and can be readily adapted to multi-column or multi-well synthesis platforms.

After completing a tandem synthesis of a siNA oligo and its complement in which the 5'-terminal dimethoxytrityl (5'-O-DMT) group remains intact (trityl on synthesis), the 5 oligonucleotides are deprotected as described above. Following deprotection, the siNA sequence strands are allowed to spontaneously hybridize. This hybridization yields a duplex in which one strand has retained the 5'-O-DMT group while the complementary strand comprises a terminal 5'-hydroxyl. The newly formed duplex behaves as a single molecule during routine solid-phase extraction purification (Trityl-On purification) even 10 though only one molecule has a dimethoxytrityl group. Because the strands form a stable duplex, this dimethoxytrityl group (or an equivalent group, such as other trityl groups or other hydrophobic moieties) is all that is required to purify the pair of oligos, for example, by using a C18 cartridge.

Standard phosphoramidite synthesis chemistry is used up to the point of 15 introducing a tandem linker, such as an inverted deoxy abasic succinate or glyceryl succinate linker (see **Figure 1**) or an equivalent cleavable linker. A non-limiting example of linker coupling conditions that can be used includes a hindered base such as diisopropylethylamine (DIPA) and/or DMAP in the presence of an activator reagent such as Bromotripyrrolidinophosphoniumhexaflurorophosphate (PyBrOP). After the linker is 20 coupled, standard synthesis chemistry is utilized to complete synthesis of the second sequence leaving the terminal the 5'-O-DMT intact. Following synthesis, the resulting oligonucleotide is deprotected according to the procedures described herein and quenched with a suitable buffer, for example with 50mM NaOAc or 1.5M NH<sub>4</sub>H<sub>2</sub>CO<sub>3</sub>.

Purification of the siNA duplex can be readily accomplished using solid phase 25 extraction, for example, using a Waters C18 SepPak 1g cartridge conditioned with 1 column volume (CV) of acetonitrile, 2 CV H<sub>2</sub>O, and 2 CV 50mM NaOAc. The sample is loaded and then washed with 1 CV H<sub>2</sub>O or 50mM NaOAc. Failure sequences are eluted with 1 CV 14% ACN (Aqueous with 50mM NaOAc and 50mM NaCl). The column is then washed, for example with 1 CV H<sub>2</sub>O followed by on-column 30 detritylation, for example by passing 1 CV of 1% aqueous trifluoroacetic acid (TFA) over the column, then adding a second CV of 1% aqueous TFA to the column and

allowing to stand for approximately 10 minutes. The remaining TFA solution is removed and the column washed with H<sub>2</sub>O followed by 1 CV 1M NaCl and additional H<sub>2</sub>O. The siNA duplex product is then eluted, for example, using 1 CV 20% aqueous CAN.

5       Figure 2 provides an example of MALDI-TOF mass spectrometry analysis of a purified siNA construct in which each peak corresponds to the calculated mass of an individual siNA strand of the siNA duplex. The same purified siNA provides three peaks when analyzed by capillary gel electrophoresis (CGE), one peak presumably corresponding to the duplex siNA, and two peaks presumably corresponding to the  
10      separate siNA sequence strands. Ion exchange HPLC analysis of the same siNA contract only shows a single peak. Testing of the purified siNA construct using a luciferase reporter assay described below demonstrated the same RNAi activity compared to siNA constructs generated from separately synthesized oligonucleotide sequence strands.

Example 2: Identification of potential siNA target sites in any RNA sequence

15       The sequence of an RNA target of interest, such as a viral or human mRNA transcript, is screened for target sites, for example by using a computer folding algorithm. In a non-limiting example, the sequence of a gene or RNA gene transcript derived from a database, such as Genbank, is used to generate siNA targets having complementarity to the target. Such sequences can be obtained from a database, or can  
20      be determined experimentally as known in the art. Target sites that are known, for example, those target sites determined to be effective target sites based on studies with other nucleic acid molecules, for example ribozymes or antisense, or those targets known to be associated with a disease or condition such as those sites containing mutations or deletions, can be used to design siNA molecules targeting those sites. Various  
25      parameters can be used to determine which sites are the most suitable target sites within the target RNA sequence. These parameters include but are not limited to secondary or tertiary RNA structure, the nucleotide base composition of the target sequence, the degree of homology between various regions of the target sequence, or the relative position of the target sequence within the RNA transcript. Based on these  
30      determinations, any number of target sites within the RNA transcript can be chosen to screen siNA molecules for efficacy, for example by using *in vitro* RNA cleavage assays,

cell culture, or animal models. In a non-limiting example, anywhere from 1 to 1000 target sites are chosen within the transcript based on the size of the siNA construct to be used. High throughput screening assays can be developed for screening siNA molecules using methods known in the art, such as with multi-well or multi-plate assays to 5 determine efficient reduction in target gene expression.

Example 3: Selection of siNA molecule target sites in a RNA

The following non-limiting steps can be used to carry out the selection of siNAs targeting a given gene sequence or transcript.

1. The target sequence is parsed *in silico* into a list of all fragments or subsequences of a 10 particular length, for example 23 nucleotide fragments, contained within the target sequence. This step is typically carried out using a custom Perl script, but commercial sequence analysis programs such as Oligo, MacVector, or the GCG Wisconsin Package can be employed as well.
2. In some instances the siNAs correspond to more than one target sequence; such 15 would be the case for example in targeting different transcripts of the same gene, targeting different transcripts of more than one gene, or for targeting both the human gene and an animal homolog. In this case, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find matching sequences in each list. The subsequences are then ranked according to the number of 20 target sequences that contain the given subsequence; the goal is to find subsequences that are present in most or all of the target sequences. Alternately, the ranking can identify subsequences that are unique to a target sequence, such as a mutant target sequence. Such an approach would enable the use of siNA to target specifically the mutant sequence and not effect the expression of the normal sequence.
- 25 3. In some instances the siNA subsequences are absent in one or more sequences while present in the desired target sequence; such would be the case if the siNA targets a gene with a paralogous family member that is to remain untargeted. As in case 2 above, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find sequences that are present in the target gene 30 but are absent in the untargeted paralog.

4. The ranked siNA subsequences can be further analyzed and ranked according to GC content. A preference can be given to sites containing 30-70% GC, with a further preference to sites containing 40-60% GC.
5. The ranked siNA subsequences can be further analyzed and ranked according to self-folding and internal hairpins. Weaker internal folds are preferred; strong hairpin structures are to be avoided.
6. The ranked siNA subsequences can be further analyzed and ranked according to whether they have runs of GGG or CCC in the sequence. GGG (or even more Gs) in either strand can make oligonucleotide synthesis problematic and can potentially interfere with RNAi activity, so it is avoided whenever better sequences are available. CCC is searched in the target strand because that will place GGG in the antisense strand.  
10
7. The ranked siNA subsequences can be further analyzed and ranked according to whether they have the dinucleotide UU (uridine dinucleotide) on the 3'-end of the sequence, and/or AA on the 5'-end of the sequence (to yield 3' UU on the antisense sequence). These sequences allow one to design siNA molecules with terminal TT thymidine dinucleotides.  
15
8. Four or five target sites are chosen from the ranked list of subsequences as described above. For example, in subsequences having 23 nucleotides, the right 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the upper (sense) strand of the siNA duplex, while the reverse complement of the left 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the lower (antisense) strand of the siNA duplex (see **Tables II and III**). If terminal TT residues are desired for the sequence (as described in paragraph 7), then the two 3' terminal nucleotides of both the sense and antisense strands are replaced by TT prior to synthesizing the oligos.  
20
9. The siNA molecules are screened in an *in vitro*, cell culture or animal model system to identify the most active siNA molecule or the most preferred target site within the target RNA sequence.  
25

10. Other design considerations can be used when selecting target nucleic acid sequences, see, for example, Reynolds *et al.*, 2004, *Nature Biotechnology Advanced Online Publication*, 1 February 2004, doi:10.1038/nbt936 and Ui-Tei *et al.*, 2004, *Nucleic Acids Research*, 32, doi:10.1093/nar/gkh247.

5        In an alternate approach, a pool of siNA constructs specific to a GPRA and/or AAA1 target sequence is used to screen for target sites in cells expressing GPRA and/or AAA1 RNA, such as such A549. The general strategy used in this approach is shown in **Figure 9**. A non-limiting example of such is a pool comprising sequences having any of SEQ ID NOS. 1-806. Cells expressing GPRA and/or AAA1 are transfected with the  
10 pool of siNA constructs and cells that demonstrate a phenotype associated with GPRA and/or AAA1 inhibition are sorted. The pool of siNA constructs can be expressed from transcription cassettes inserted into appropriate vectors (see for example **Figure 7** and **Figure 8**). The siNA from cells demonstrating a positive phenotypic change (e.g., decreased proliferation, decreased GPRA and/or AAA1 mRNA levels or decreased  
15 GPRA and/or AAA1 protein expression), are sequenced to determine the most suitable target site(s) within the target GPRA and/or AAA1 RNA sequence.

Example 4: GPRA and/or AAA1 targeted siNA design

siNA target sites were chosen by analyzing sequences of the GPRA and/or AAA1 RNA target and optionally prioritizing the target sites on the basis of folding (structure of  
20 any given sequence analyzed to determine siNA accessibility to the target), by using a library of siNA molecules as described in Example 3, or alternately by using an *in vitro* siNA system as described in Example 6 herein. siNA molecules were designed that could bind each target and are optionally individually analyzed by computer folding to assess whether the siNA molecule can interact with the target sequence. Varying the  
25 length of the siNA molecules can be chosen to optimize activity. Generally, a sufficient number of complementary nucleotide bases are chosen to bind to, or otherwise interact with, the target RNA, but the degree of complementarity can be modulated to accommodate siNA duplexes or varying length or base composition. By using such methodologies, siNA molecules can be designed to target sites within any known RNA  
30 sequence, for example those RNA sequences corresponding to the any gene transcript.

Chemically modified siNA constructs are designed to provide nuclease stability for systemic administration *in vivo* and/or improved pharmacokinetic, localization, and delivery properties while preserving the ability to mediate RNAi activity. Chemical modifications as described herein are introduced synthetically using synthetic methods 5 described herein and those generally known in the art. The synthetic siNA constructs are then assayed for nuclease stability in serum and/or cellular/tissue extracts (e.g. liver extracts). The synthetic siNA constructs are also tested in parallel for RNAi activity using an appropriate assay, such as a luciferase reporter assay as described herein or another suitable assay that can quantity RNAi activity. Synthetic siNA constructs that 10 possess both nuclease stability and RNAi activity can be further modified and re-evaluated in stability and activity assays. The chemical modifications of the stabilized active siNA constructs can then be applied to any siNA sequence targeting any chosen RNA and used, for example, in target screening assays to pick lead siNA compounds for therapeutic development (see for example **Figure 11**).

15 **Example 5: Chemical Synthesis and Purification of siNA**

siNA molecules can be designed to interact with various sites in the RNA message, for example, target sequences within the RNA sequences described herein. The sequence of one strand of the siNA molecule(s) is complementary to the target site sequences described above. The siNA molecules can be chemically synthesized using 20 methods described herein. Inactive siNA molecules that are used as control sequences can be synthesized by scrambling the sequence of the siNA molecules such that it is not complementary to the target sequence. Generally, siNA constructs can be synthesized using solid phase oligonucleotide synthesis methods as described herein (see for example Usman *et al.*, US Patent Nos. 5,804,683; 5,831,071; 5,998,203; 6,117,657; 6,353,098; 25 6,362,323; 6,437,117; 6,469,158; Scaringe *et al.*, US Patent Nos. 6,111,086; 6,008,400; 6,111,086 all incorporated by reference herein in their entirety).

In a non-limiting example, RNA oligonucleotides are synthesized in a stepwise fashion using the phosphoramidite chemistry as is known in the art. Standard phosphoramidite chemistry involves the use of nucleosides comprising any of 5'-O-30 dimethoxytrityl, 2'-O-tert-butyldimethylsilyl, 3'-O-2-Cyanoethyl N,N-diisopropylphosphoroamidite groups, and exocyclic amine protecting groups (e.g. N6-benzoyl adenosine,

N4 acetyl cytidine, and N2-isobutyryl guanosine). Alternately, 2'-O-Silyl Ethers can be used in conjunction with acid-labile 2'-O-orthoester protecting groups in the synthesis of RNA as described by Scaringe *supra*. Differing 2' chemistries can require different protecting groups, for example 2'-deoxy-2'-amino nucleosides can utilize N-phthaloyl 5 protection as described by Usman *et al.*, US Patent 5,631,360, incorporated by reference herein in its entirety).

During solid phase synthesis, each nucleotide is added sequentially (3' to 5'-direction) to the solid support-bound oligonucleotide. The first nucleoside at the 3'-end of the chain is covalently attached to a solid support (e.g., controlled pore glass or 10 polystyrene) using various linkers. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator are combined resulting in the coupling of the second nucleoside phosphoramidite onto the 5'-end of the first nucleoside. The support is then washed and any unreacted 5'-hydroxyl groups are capped with a capping reagent such as acetic anhydride to yield inactive 5'-acetyl moieties. The trivalent phosphorus linkage is 15 then oxidized to a more stable phosphate linkage. At the end of the nucleotide addition cycle, the 5'-O-protecting group is cleaved under suitable conditions (e.g., acidic conditions for trityl-based groups and Fluoride for silyl-based groups). The cycle is repeated for each subsequent nucleotide.

Modification of synthesis conditions can be used to optimize coupling efficiency, 20 for example by using differing coupling times, differing reagent/phosphoramidite concentrations, differing contact times, differing solid supports and solid support linker chemistries depending on the particular chemical composition of the siNA to be synthesized. Deprotection and purification of the siNA can be performed as is generally described in Usman *et al.*, US 5,831,071, US 6,353,098, US 6,437,117, and Bellon *et al.*, 25 US 6,054,576, US 6,162,909, US 6,303,773, or Scaringe *supra*, incorporated by reference herein in their entireties. Additionally, deprotection conditions can be modified to provide the best possible yield and purity of siNA constructs. For example, applicant has observed that oligonucleotides comprising 2'-deoxy-2'-fluoro nucleotides can degrade under inappropriate deprotection conditions. Such oligonucleotides are 30 deprotected using aqueous methylamine at about 35°C for 30 minutes. If the 2'-deoxy-2'-fluoro containing oligonucleotide also comprises ribonucleotides, after deprotection

with aqueous methylamine at about 35°C for 30 minutes, TEA-HF is added and the reaction maintained at about 65°C for an additional 15 minutes.

Example 6: RNAi *in vitro* assay to assess siNA activity

An *in vitro* assay that recapitulates RNAi in a cell-free system is used to evaluate 5 siNA constructs targeting GPRA and/or AAA1 RNA targets. The assay comprises the system described by Tuschl *et al.*, 1999, *Genes and Development*, 13, 3191-3197 and Zamore *et al.*, 2000, *Cell*, 101, 25-33 adapted for use with GPRA and/or AAA1 target RNA. A *Drosophila* extract derived from syncytial blastoderm is used to reconstitute RNAi activity *in vitro*. Target RNA is generated via *in vitro* transcription from an 10 appropriate GPRA and/or AAA1 expressing plasmid using T7 RNA polymerase or via chemical synthesis as described herein. Sense and antisense siNA strands (for example 20 uM each) are annealed by incubation in buffer (such as 100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 1 minute at 90°C followed by 1 hour at 37°C, then diluted in lysis buffer (for example 100 mM potassium acetate, 30 15 mM HEPES-KOH at pH 7.4, 2mM magnesium acetate). Annealing can be monitored by gel electrophoresis on an agarose gel in TBE buffer and stained with ethidium bromide. The *Drosophila* lysate is prepared using zero to two-hour-old embryos from Oregon R flies collected on yeasted molasses agar that are dechorionated and lysed. The lysate is centrifuged and the supernatant isolated. The assay comprises a reaction mixture 20 containing 50% lysate [vol/vol], RNA (10-50 pM final concentration), and 10% [vol/vol] lysis buffer containing siNA (10 nM final concentration). The reaction mixture also contains 10 mM creatine phosphate, 10 ug/ml creatine phosphokinase, 100 um GTP, 100 uM UTP, 100 uM CTP, 500 uM ATP, 5 mM DTT, 0.1 U/uL RNasin (Promega), and 100 uM of each amino acid. The final concentration of potassium acetate is adjusted to 100 25 mM. The reactions are pre-assembled on ice and preincubated at 25° C for 10 minutes before adding RNA, then incubated at 25° C for an additional 60 minutes. Reactions are quenched with 4 volumes of 1.25 x Passive Lysis Buffer (Promega). Target RNA cleavage is assayed by RT-PCR analysis or other methods known in the art and are compared to control reactions in which siNA is omitted from the reaction.

30 Alternately, internally-labeled target RNA for the assay is prepared by *in vitro* transcription in the presence of [ $\alpha$ -<sup>32</sup>P] CTP, passed over a G50 Sephadex column by

spin chromatography and used as target RNA without further purification. Optionally, target RNA is 5'-<sup>32</sup>P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed as described above and target RNA and the specific RNA cleavage products generated by RNAi are visualized on an autoradiograph of a gel. The percentage of 5 cleavage is determined by PHOSPHOR IMAGER® (autoradiography) quantitation of bands representing intact control RNA or RNA from control reactions without siNA and the cleavage products generated by the assay.

In one embodiment, this assay is used to determine target sites in the GPRA and/or AAA1 RNA target for siNA mediated RNAi cleavage, wherein a plurality of siNA 10 constructs are screened for RNAi mediated cleavage of the GPRA and/or AAA1 RNA target, for example, by analyzing the assay reaction by electrophoresis of labeled target RNA, or by northern blotting, as well as by other methodology well known in the art.

Example 7: Nucleic acid inhibition of GPRA and/or AAA1 target RNA

siNA molecules targeted to the human GPRA and/or AAA1 RNA are designed and 15 synthesized as described above. These nucleic acid molecules can be tested for cleavage activity *in vivo*, for example, using the following procedure. The target sequences and the nucleotide location within the GPRA and/or AAA1 RNA are given in **Tables II and III**.

Two formats are used to test the efficacy of siNAs targeting GPRA and/or AAA1. 20 First, the reagents are tested in cell culture using, for example, A549 cells, to determine the extent of RNA and protein inhibition. siNA reagents (*e.g.*; see **Tables II and III**) are selected against the GPRA and/or AAA1 target as described herein. RNA inhibition is measured after delivery of these reagents by a suitable transfection agent to, for example, cultured A549 cells. Relative amounts of target RNA are measured versus actin using 25 real-time PCR monitoring of amplification (*eg.*, ABI 7700 TAQMAN®). A comparison is made to a mixture of oligonucleotide sequences made to unrelated targets or to a randomized siNA control with the same overall length and chemistry, but randomly substituted at each position. Primary and secondary lead reagents are chosen for the target and optimization performed. After an optimal transfection agent concentration is

chosen, a RNA time-course of inhibition is performed with the lead siNA molecule. In addition, a cell-plating format can be used to determine RNA inhibition.

Delivery of siNA to Cells

Cells such as A549 cells are seeded, for example, at  $1 \times 10^5$  cells per well of a six-well dish in EGM-2 (BioWhittaker) the day before transfection. siNA (final concentration, for example 20nM) and cationic lipid (e.g., final concentration 2 $\mu$ g/ml) are complexed in EGM basal media (Bio Whittaker) at 37°C for 30 minutes in polystyrene tubes. Following vortexing, the complexed siNA is added to each well and incubated for the times indicated. For initial optimization experiments, cells are seeded, for example, at  $1 \times 10^3$  in 96 well plates and siNA complex added as described. Efficiency of delivery of siNA to cells is determined using a fluorescent siNA complexed with lipid. Cells in 6-well dishes are incubated with siNA for 24 hours, rinsed with PBS and fixed in 2% paraformaldehyde for 15 minutes at room temperature. Uptake of siNA is visualized using a fluorescent microscope.

15 TAQMAN® (real-time PCR monitoring of amplification) and Lightcycler quantification of mRNA

Total RNA is prepared from cells following siNA delivery, for example, using Qiagen RNA purification kits for 6-well or Rneasy extraction kits for 96-well assays. For TAQMAN® analysis (real-time PCR monitoring of amplification), dual-labeled probes are synthesized with the reporter dye, FAM or JOE, covalently linked at the 5'-end and the quencher dye TAMRA conjugated to the 3'-end. One-step RT-PCR amplifications are performed on, for example, an ABI PRISM 7700 Sequence Detector using 50  $\mu$ l reactions consisting of 10  $\mu$ l total RNA, 100 nM forward primer, 900 nM reverse primer, 100 nM probe, 1X TaqMan PCR reaction buffer (PE-Applied Biosystems), 5.5 mM MgCl<sub>2</sub>, 300  $\mu$ M each dATP, dCTP, dGTP, and dTTP, 10U RNase Inhibitor (Promega), 1.25U AMPLITAQ GOLD® (DNA polymerase) (PE-Applied Biosystems) and 10U M-MLV Reverse Transcriptase (Promega). The thermal cycling conditions can consist of 30 minutes at 48°C, 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Quantitation of mRNA levels is determined relative to standards generated from serially diluted total cellular RNA (300, 100, 33, 11 ng/rxn) and

normalizing to  $\beta$ -actin or GAPDH mRNA in parallel TAQMAN® reactions (real-time PCR monitoring of amplification). For each gene of interest an upper and lower primer and a fluorescently labeled probe are designed. Real time incorporation of SYBR Green I dye into a specific PCR product can be measured in glass capillary tubes using a 5 lightcyler. A standard curve is generated for each primer pair using control cRNA. Values are represented as relative expression to GAPDH in each sample.

#### Western blotting

Nuclear extracts can be prepared using a standard micro preparation technique (see for example Andrews and Faller, 1991, *Nucleic Acids Research*, 19, 2499). Protein 10 extracts from supernatants are prepared, for example using TCA precipitation. An equal volume of 20% TCA is added to the cell supernatant, incubated on ice for 1 hour and pelleted by centrifugation for 5 minutes. Pellets are washed in acetone, dried and resuspended in water. Cellular protein extracts are run on a 10% Bis-Tris NuPage (nuclear extracts) or 4-12% Tris-Glycine (supernatant extracts) polyacrylamide gel and 15 transferred onto nitro-cellulose membranes. Non-specific binding can be blocked by incubation, for example, with 5% non-fat milk for 1 hour followed by primary antibody for 16 hour at 4°C. Following washes, the secondary antibody is applied, for example (1:10,000 dilution) for 1 hour at room temperature and the signal detected with SuperSignal reagent (Pierce).

20 Example 8: Animal Models useful to evaluate the down-regulation of GPRA and/or AAA1 gene expression

Evaluating the efficacy of anti-GPRA and/or AAA1 agents in animal models is an important prerequisite to human clinical trials. Laitinen *et al.*, 2004, *Science*, 304, 300-304, describe a mouse model of ovalbumin-induced lung inflammation in which 25 GPRA mRNA is significantly up-regulated in mouse lung after ovalbumin tests in sensitized compared with nonsensitized mice. Using this model, ovalbumin sensitized mice can be treated with active and control siNA molecules of the invention and GPRA mRNA and/or protein levels can be assayed to identify or validate efficacious siNA molecules of the invention that are useful in treating asthma and other conditions that

respond to GPRA or AAA1. As such, this model provides an animal model for testing therapeutic drugs, including siNA constructs of the instant invention.

Example 9: RNAi mediated inhibition of GPRA and/or AAA1 expression

siNA constructs (**Table III**) are tested for efficacy in reducing GPRA and/or AAA1 RNA expression in, for example, A549 cells. Cells are plated approximately 24 hours before transfection in 96-well plates at 5,000-7,500 cells/well, 100  $\mu$ l/well, such that at the time of transfection cells are 70-90% confluent. For transfection, annealed siNAs are mixed with the transfection reagent (Lipofectamine 2000, Invitrogen) in a volume of 50  $\mu$ l/well and incubated for 20 minutes at room temperature. The siNA transfection mixtures are added to cells to give a final siNA concentration of 25 nM in a volume of 150  $\mu$ l. Each siNA transfection mixture is added to 3 wells for triplicate siNA treatments. Cells are incubated at 37° for 24 hours in the continued presence of the siNA transfection mixture. At 24 hours, RNA is prepared from each well of treated cells. The supernatants with the transfection mixtures are first removed and discarded, then the cells are lysed and RNA prepared from each well. Target gene expression following treatment is evaluated by RT-PCR for the target gene and for a control gene (36B4, an RNA polymerase subunit) for normalization. The triplicate data is averaged and the standard deviations determined for each treatment. Normalized data are graphed and the percent reduction of target mRNA by active siNAs in comparison to their respective inverted control siNAs is determined.

Example 10: Indications

The present body of knowledge in GPRA and/or AAA1 research indicates the need for methods to assay GPRA and/or AAA1 activity and for compounds that can regulate GPRA and/or AAA1 expression for research, diagnostic, and therapeutic use. As described herein, the nucleic acid molecules of the present invention can be used in assays to diagnose disease state related of GPRA and/or AAA1 levels. In addition, the nucleic acid molecules can be used to treat disease state related to GPRA and/or AAA1 levels.

Particular conditions and disease states that can be associated with GPRA and/or AAA1 expression modulation include, but are not limited to asthma, chronic obstructive

pulmonary disease or "COPD", allergic rhinitis, sinusitis, pulmonary vasoconstriction, inflammation, allergies, impeded respiration, respiratory distress syndrome, cystic fibrosis, pulmonary hypertension, pulmonary vasoconstriction, emphysema, and any other diseases or conditions related to asthma that are related to or will respond to the 5 levels of a GPRA and/or AAA1 gene in a cell or tissue, alone or in combination with other therapies.

The use of anticholinergic agents, anti-inflammatories, bronchodilators, adenosine inhibitors, adenosine A1 receptor inhibitors, non-selective M3 receptor antagonists such as atropine, ipratropium bromide and selective M3 receptor antagonists such as 10 darifenacin and revatropate are all non-limiting examples of agents that can be combined with or used in conjunction with the nucleic acid molecules (e.g. siNA molecules) of the instant invention. Those skilled in the art will recognize that other compounds and therapies used to treat the diseases and conditions described herein can similarly be combined with the nucleic acid molecules of the instant invention (e.g. siNA molecules) 15 and are hence within the scope of the instant invention.

#### Example 11: Diagnostic uses

The siNA molecules of the invention can be used in a variety of diagnostic applications, such as in the identification of molecular targets (e.g., RNA) in a variety of applications, for example, in clinical, industrial, environmental, agricultural and/or 20 research settings. Such diagnostic use of siNA molecules involves utilizing reconstituted RNAi systems, for example, using cellular lysates or partially purified cellular lysates. siNA molecules of this invention can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of endogenous or exogenous, for example viral, RNA in a cell. The close relationship between siNA 25 activity and the structure of the target RNA allows the detection of mutations in any region of the molecule, which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple siNA molecules described in this invention, one can map nucleotide changes, which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with siNA molecules can be used 30 to inhibit gene expression and define the role of specified gene products in the progression of disease or infection. In this manner, other genetic targets can be defined

as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple siNA molecules targeted to different genes, siNA molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations siNA 5 molecules and/or other chemical or biological molecules). Other *in vitro* uses of siNA molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with a disease, infection, or related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a siNA using standard methodologies, for example, fluorescence resonance emission 10 transfer (FRET).

In a specific example, siNA molecules that cleave only wild-type or mutant forms of the target RNA are used for the assay. The first siNA molecules (*i.e.*, those that cleave only wild-type forms of target RNA) are used to identify wild-type RNA present in the sample and the second siNA molecules (*i.e.*, those that cleave only mutant forms 15 of target RNA) are used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both siNA molecules to demonstrate the relative siNA efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and 20 mutant RNAs in the sample population. Thus, each analysis requires two siNA molecules, two substrates and one unknown sample, which is combined into six reactions. The presence of cleavage products is determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain 25 insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, disease related or infection related) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels is adequate and decreases the cost of the 30 initial diagnosis. Higher mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

5       One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to  
10      those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the  
15      scope of the present invention and the following claims. The present invention teaches one skilled in the art to test various combinations and/or substitutions of chemical modifications described herein toward generating nucleic acid constructs with improved activity for mediating RNAi activity. Such improved activity can comprise improved stability, improved bioavailability, and/or improved activation of cellular responses  
20      mediating RNAi. Therefore, the specific embodiments described herein are not limiting and one skilled in the art can readily appreciate that specific combinations of the modifications described herein can be tested without undue experimentation toward identifying siNA molecules with improved RNAi activity.

25      The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of  
30      such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible

within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be

5 within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

**Table I: GPRA and AAA1 Accession Numbers**

NM\_207172  
5 Homo sapiens G protein-coupled receptor 154 (GPR154), transcript variant 1, mRNA  
gi|46395495|ref|NM\_207172.1|[46395495]

NM\_207173  
10 Homo sapiens G protein-coupled receptor 154 (GPR154), transcript variant 2, mRNA  
gi|46391084|ref|NM\_207173.1|[46391084]

NM\_207284  
15 Homo sapiens AAA1 protein (AAA1), transcript variant II, mRNA  
gi|46402493|ref|NM\_207284.1|[46402493]

NM\_207285  
20 Homo sapiens AAA1 protein (AAA1), transcript variant III, mRNA  
gi|46402501|ref|NM\_207285.1|[46402501]

NM\_207286  
25 Homo sapiens AAA1 protein (AAA1), transcript variant IV, mRNA  
gi|46402497|ref|NM\_207286.1|[46402497]

NM\_207287  
30 Homo sapiens AAA1 protein (AAA1), transcript variant V, mRNA  
gi|46402505|ref|NM\_207287.1|[46402505]

NM\_207288  
35 Homo sapiens AAA1 protein (AAA1), transcript variant VI, mRNA  
gi|46402499|ref|NM\_207288.1|[46402499]

NM\_207289  
40 Homo sapiens AAA1 protein (AAA1), transcript variant VII, mRNA  
gi|46402508|ref|NM\_207289.1|[46402508]

NM\_207290  
45 Homo sapiens AAA1 protein (AAA1), transcript variant VIII, mRNA  
gi|46402503|ref|NM\_207290.1|[46402503]

NM\_207283

Homo sapiens AAA1 protein (AAA1), transcript variant IX,  
mRNA  
gi|46402495|ref|NM\_207283.1|[46402495]

5

TABLE II: GPR154-1, GPR154-2, AAA1-2, AAA1-3, AAA1-4, AAA1-5, AAA1-6, AAA1-7, AAA1-8, AAA1-9  
SINA AND TARGET SEQUENCES

## GPR154-1 NM\_207172

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3	GCUAGGGGGCUCUGUG	1	3	GCUCAGGGGGCUCUGUG	1	21	CACAGGCCUCUCUGAGC	88
21	GCCUCGGUUACGGAGCU	2	21	GCCUCGGUUACGGAGCU	2	39	AGCUCUGCUGAACGGAGGC	89
39	UGCAGCUGCUGGCCAGCUC	3	39	UGCAGCUGCUGGCCAGCUC	3	57	GAGCJUGGGCAGCAGCUGCA	90
57	CUCAGGAGGCAAGCUGGAC	4	57	CUCAGGAGGCAAGCUGGAC	4	75	GUCCAGCUUCCUCUGAG	91
75	CUCCCUCACUCAGCUGGAG	5	75	CUCCCUCACUCAGCUGGAG	5	93	CUGCAGCUGAGUGAGGGAG	92
93	GGAGCAAGGACAGUGAGGC	6	93	GGAGCAAGGACAGUGAGGC	6	111	GCCUACUGUCCUUCGUCC	93
111	CUCAACCCCGCCUGAGCCA	7	111	CUCAACCCCGCCUGAGCCA	7	129	UGGCUCAGGGGGGUUGAG	94
129	AUGCCAGCCACUUCACAG	8	129	AUGCCAGCCACUUCACAG	8	147	CUGUGAAGUUGGCGUGGCAU	95
147	GAGGGCAGCUCUGCAGUCCA	9	147	GAGGGCAGCUCUGCAGUCCA	9	165	UGGAAUCGAAGCUGGCCUC	96
165	AGUGGGACCGGGCAGACGC	10	165	AGUGGGACCGGGCAGACGC	10	183	GCGUGUCGCCGGGUCCCCACU	97
183	CUGGAUUCUCCCCAGUUG	11	183	CUGGAUUCUCCCCAGUUG	11	201	CCACUGGGGAAGAAUCCAG	98
201	GCUUJGCACUGAAACAGUGA	12	201	GCUUJGCACUGAAACAGUGA	12	219	UCACJGUUUCAGUGCAAGC	99
219	ACUUUUACUGAAGUGGGUG	13	219	ACUUUUACUGAAGUGGGUG	13	237	CCACCCACUUCAGUAAAAGU	100
237	GAAGGAAAAGGAUUGGGGU	14	237	GAAGGAAAAGGAUUGGGGU	14	255	AACCCCAUUCUUCUUCUUC	101
255	UCCUUCUACUACUCCUUA	15	255	UCCUUCUACUACUCCUUA	15	273	UAAAAGGAGUAGUAGAAGGA	102
273	AAGACUGAGCAAUUGAUAA	16	273	AAGACUGAGCAAUUGAUAA	16	291	UUUAUCAAUUGCUCAGUCUU	103
291	ACUCUGGGGUCCUCUUG	17	291	ACUCUGGGGUCCUCUUG	17	309	CAAAGAGGACCCACAGAGU	104
309	GUUUUUACCAUJGUUGGAA	18	309	GUUUUUACCAUJGUUGGAA	18	327	UUCCAACAAUUGGAAAAC	105
327	AACUCUCCGUUGUGGUUUUU	19	327	AACUCUCCGUUGUGGUUUUU	19	345	AAAAAAAGCACAAACGGAGGU	106
345	UCCACAUAGGAGGAAGA	20	345	UCCACAUAGGAGGAAGA	20	363	UCUUIUCUCCAUUGGGA	107
363	AAGAAAGCUAAGAAUAGACCU	21	363	AAGAAAGCUAAGAAUAGACCU	21	381	AGGUCAUUCUJGACUUCUU	108
381	UUCUUUUGAGACUGCUGG	22	381	UUCUUUUGAGACUGCUGG	22	399	CCAGCUGAGUACAAAGAA	109
399	GCCAUACAGAUUCUUCUCA	23	399	GCCAUACAGAUUCUUCUCA	23	417	UGAAAGAAUUCUGUGAUGGC	110
417	ACAGGGACUGGUCAACAUU	24	417	ACAGGGACUGGUCAACAUU	24	435	AGAUGUUGACCCAGUCCUGU	111
435	UUGACAGAUUJAAUUGGC	25	435	UUGACAGAUUJAAUUGGC	25	453	GCCAAUAAAUAUCUGUCAA	112
453	CGAUUJCACUGGAGACUUC	26	453	CGAUUJCACUGGAGACUUC	26	471	UGAAGUUCUCCAGUGAAUCG	113
471	ACGGGACCCUGACCUGGUU	27	471	ACGGGACCCUGACCUGGUU	27	489	AAACCAGGUACGGUGCCGU	114
489	UGCCGAGGGGUCCGGCUAU	28	489	UGCCGAGGGGUCCGGCUAU	28	507	AAUAGGGGACCCACUGGCA	115

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
507	UUGCAGGUUGUGUGCUU	29	507	UUGCAGGUUGUGUGCUU	29	525	AGAGCAGCACAAACCUGCAA	116
525	UACGCCUCUACCUACGUCC	30	525	UACGCCUCUACCUACGUCC	30	543	GGACGUAGGUAGGGCGUA	117
543	CUGGUGCCUCAGCAUAG	31	543	CUGGUGCCUCAGCAUAG	31	561	CUAUGCUGAGGGACACCAG	118
561	GACAGAUACCAUGCCAUAG	32	561	GACAGAUACCAUGCCAUAG	32	579	CGAUGCAUGGUACUGUC	119
579	GUCUACCCCAUGAAGUUC	33	579	GUCUACCCCAUGAAGUUC	33	597	GGAAUCUCAUGGGGUAGAC	120
597	CUUCAAGGAGAAAAGCAAG	34	597	CUUCAAGGAGAAAAGCAAG	34	615	CUUGGUUUUCUCCUUGAAG	121
615	GCCAGGGGUCCUCAUUGUGA	35	615	GCCAGGGGUCCUCAUUGUGA	35	633	UCACAUAGGACCUCUGGC	122
633	AUCGCCUGGAGGCCUGUU	36	633	AUCGCCUGGAGGCCUGUU	36	651	AAGACAGGUCCAGGGGAGU	123
651	UUUCUGUUCUCCAUUCCCA	37	651	UUUCUGUUCUCCAUUCCCA	37	669	UGGAAUUGGAGAACAGAAA	124
669	ACCCUGAUCAUJJUGGA	38	669	ACCCUGAUCAUJJUGGA	38	687	UCCCAAUAUUGAUCAAGGGU	125
687	AAGAGGACACGUCCAAACG	39	687	AAGAGGACACGUCCAAACG	39	705	CGUUGGACAGGUCCUCUU	126
705	GGUGAAGUGGAGUGUGGG	40	705	GGUGAAGUGGAGUGUGGG	40	723	CCCAGCACUGGCACUUCACC	127
723	GCCCCUGGGCCUGACGACU	41	723	GCCCCUGGGCCUGACGACU	41	741	AGUCGUAGGCCACAGGGC	128
741	UCCUACUGGACCCAUACA	42	741	UCCUACUGGACCCAUACA	42	759	UGUAUGGGGUCCAGUAGGA	129
759	AUGACCAUCUGGGCUUCC	43	759	AUGACCAUCUGGGCUUCC	43	777	GGAAAGGCCACGAUGGUCAU	130
777	CUGGUGUACUCAUCCUC	44	777	CUGGUGUACUCAUCCUC	44	795	GAGGGAGAAGUACACCAG	131
795	CUGACAAUCAUCAGCAUCA	45	795	CUGACAAUCAUCAGCAUCA	45	813	UGAUGCUGAUGAUUGUCAG	132
813	AUGUAUGGGCAUUGGAUCC	46	813	AUGUAUGGGCAUUGGAUCC	46	831	GGAUCAACAAUGCCAUACAU	133
831	CGAACUAAUUGGAAUAAA	47	831	CGAACUAAUUGGAAUAAA	47	849	UUUUAAUCCAUAUAGUUCG	134
849	AGCAAAACCUACGAAACAG	48	849	AGCAAAACCUACGAAACAG	48	867	CUGUUUCGUAGGUUUUGCU	135
867	GUGAUUUCCAACUGUCUAG	49	867	GUGAUUUCCAACUGUCUAG	49	885	CUGAGCAGUUGGAAUACAC	136
885	GAUGGGAAACUGUGGAGCA	50	885	GAUGGGAAACUGUGGAGCA	50	903	UGCUCACAGUUCUCCAU	137
903	AGCUAUACCGAGGACUCA	51	903	AGCUAUACCGAGGACUCA	51	921	UGAGGUCCUCGGUUAUGCU	138
921	AUCUCAAAAGGCAAAAUCA	52	921	AUCUCAAAAGGCAAAAUCA	52	939	UGAUUUUUGCCUUGAGAU	139
939	AAGGCUAUCAAGUUAUGCA	53	939	AAGGCUAUCAAGUUAUGCA	53	957	UGCUUAUACUJUGAUAGCCUU	140
957	AUCAUCAUCAUUCJUGCCU	54	957	AUCAUCAUCAUUCJUGCCU	54	975	AGGCAAGAAUUGAUUGAU	141
975	UUCAUUCUGCUGUUGGAGUC	55	975	UUCAUUCUGCUGUUGGAGUC	55	993	GACUCCAACAGCAGAUGAA	142
993	CCAUACUUCUJUGUJGACA	56	993	CCAUACUUCUJUGUJGACA	56	1011	UGUCAAACAGGAAGUAGUG	143
1011	AUUUUGGACAAUJUCAAC	57	1011	AUUUUGGACAAUJUCAAC	57	1029	GGUUGAAAUUGUCCAAAAU	144
1029	CUCCUUCAGACACCCAGG	58	1029	CUCCUUCAGACACCCAGG	58	1047	CCUGGGUGUCUGGAAGGG	145
1047	GAGGGUUCUAGGCCUCUG	59	1047	GAGGGUUCUAGGCCUCUG	59	1065	CAGAGGCAUAGAAAACGCUC	146
1065	GUGAUCAUCAUCAACCUCG	60	1065	GUGAUCAUCAUCAACCUCG	60	1083	GCAGGUUCUCAUAGAUCA	147
1083	CCAGCAUUGAAUAGUGCCA	61	1083	CCAGCAUUGAAUAGUGCCA	61	1101	UGGCCACAUUCAUAGUGGG	148

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
1101	AUCAACCCCCUCAUCUACU	62	1101	AUCAACCCCCUCAUCUACU	62	1119	AGUAGAUGAGGGGGUUGAU	149
1119	UGUGUCUUCAGCAGCUCA	63	1119	UGUGUCUUCAGCAGCUCA	63	1137	UGGAGCUGCUGAAGACACA	150
1137	AUCUCUUUCCCCUGCAGGG	64	1137	AUCUCUUUCCCCUGCAGGG	64	1155	CCCUGGAGGGAAAGAGAGAU	151
1155	GAGCAAAGAUACACAGGAU	65	1155	GAGCAAAGAUACACAGGAU	65	1173	AAUCCUGUGAUUUUCGUC	152
1173	UCCAGAAUAGACGUUCCGGG	66	1173	UCCAGAAUAGACGUUCCGGG	66	1191	CCCGAACGUCAUUCUGGA	153
1191	GAGAGAACUGAGGGCAUG	67	1191	GAGAGAACUGAGGGCAUG	67	1209	CAUGCCUCUCAGUUUCUC	154
1209	GAGAUGCAGAUUCGUCCA	68	1209	GAGAUGCAGAUUCGUCCA	68	1227	UGGACAGAAUCUGCAUCUC	155
1227	AAGCCAGAAUUCAUCAUAGA	69	1227	AAGCCAGAAUUCAUCAUAGA	69	1245	UCUAGAUGAAUUCUGGUU	156
1245	ACCCUAGGGCAGUGCCAGU	70	1245	ACCCUAGGGCAGUGCCAGU	70	1263	ACUGGCACUGGCCUAGGGU	157
1263	UGCUUAGGCUGAGGCCAUC	71	1263	UGCUUAGGCUGAGGCCAUC	71	1281	GAUGGUGCUCAGGCCUAGCA	158
1281	CAGCUUCUCCAGGUCCUUG	72	1281	CAGCUUCUCCAGGUCCUUG	72	1299	CAAGGACCUGGGAGGAGCUG	159
1299	GUCACCUGCUUGGGCACGU	73	1299	GUCACCUGCUUGGGCACGU	73	1317	ACGUGGCCAAGCAGGUGGAC	160
1317	UGCAUGGAACCCGGAGCCAA	74	1317	UGCAUGGAACCCGGAGCCAA	74	1335	UUGGCUUCCGGGUUCCAUAGCA	161
1335	ACUUUACCCCCACCCUCGUC	75	1335	ACUUUACCCCCACCCUCGUC	75	1353	GACGAGGGGGGGGUGAAGU	162
1353	CAUUUACCCUGGGAGAUGCAC	76	1353	CAUUUACCCUGGGAGAUGCAC	76	1371	GUGCAUCUCCAGGUAAUG	163
1371	CAAGACAAAUGUUCUAAUG	77	1371	CAAGACAAAUGUUCUAAUG	77	1389	CAUUAAGAACAUUUCGUUUG	164
1389	GACUGCAUGCACUGCUAAA	78	1389	GACUGCAUGCACUGCUAAA	78	1407	UUAAGCAUGGCAUGCAGUC	165
1407	AGUAUUGCCAAACAGGAAC	79	1407	AGUAUUGCCAAACAGGAAC	79	1425	GUUCUGGUUGGCCAAUACU	166
1425	CUCCCAGUUAAUCAUGCC	80	1425	CUCCCAGUUAAUCAUGCC	80	1443	GGCAUQAUAUACUGGGGAG	167
1443	CAGCCAGGAAGGAAACGCC	81	1443	CAGCCAGGAAGGAAACGCC	81	1461	GGCGUUIUCCUUCCUGGCUG	168
1461	CUUCCUUCCCCACCAUUC	82	1461	CUUCCUUCCCCACCAUUC	82	1479	GGAAUGGGGGGGAAAGGAAG	169
1479	CCAGCCCCUCCUCCACUG	83	1479	CCAGCCCCUCCUCCACUG	83	1497	CAGUGGGAAAGGGGGCUGG	170
1497	GGCCAGCACCUGAACCCAG	84	1497	GGCCAGCACCUGAACCCAG	84	1515	CUGGGGUUCAGGGUGGGCC	171
1515	GUGAACACAGGCCAUUAGU	85	1515	GUGAACACAGGCCAUUAGU	85	1533	CACUAAUGCCUGGUUCAC	172
1533	GGUCCAGGGGUCCUGGCCU	86	1533	GGUCCAGGGGUCCUGGCCU	86	1551	CAAGCCAGGACCCUGGACC	173
1547	GUUUGGAGCCAGUGAGUAG	87	1547	GUUUGGAGCCAGUGAGUAG	87	1565	CUACUCACUGGCCUCCAAGC	174

## GPR154-2 NM\_207173

Pos	Seq	Seq ID	Up pos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3	GCUAAGGGAGGGCUCUGUG	1	3	GCUAAGGGAGGGCUCUGUG	1	21	CACAGAGGCCUCCCCUGAGC	88
21	GCCUCCGUUCAAGCAGGU	2	21	GCCUCCGUUCAAGCAGGU	2	39	AGCUCUJGCUGAACGGAGGC	89
39	UGGAGGUGGUGGCCAGGUC	3	39	UGGAGGUGGUGGCCAGGUC	3	57	GAGCUGGGCAGGAGCCUGGA	90
57	CUCAGGAGGCAAGCUGGAC	4	57	CUCAGGAGGCAAGCUGGAC	4	75	GUCCAGCUUGCCUCCUGAG	91
75	CUCCUCACUCAGCUGGAG	5	75	CUCCUCACUCAGCUGGAG	5	93	CUGCAGCUGAGGAGGGGAG	92
93	GGAGCAAGGACAGUGAGGC	6	93	GGAGCAAGGACAGUGAGGC	6	111	GCCUCACUGGUUJGCUCC	93
111	CUAAACCCGGCCUGAGCCA	7	111	CUAAACCCGGCCUGAGCCA	7	129	UGGCUCAGGGGGGUUGAG	94
129	AUGCCAGCCAACUUCACAG	8	129	AUGCCAGCCAACUUCACAG	8	147	CUGUGAAGUUGGUGGCCAU	95
147	GAGGGCAGCUUCGAAUCCA	9	147	GAGGGCAGCUUCGAAUCCA	9	165	UGGAUJCAGGCUGGCCUC	96
165	AGUGGGACCGGGCAGACGC	10	165	AGUGGGACCGGGCAGACGC	10	183	GCGUCUJGCCGGGUCCCCACU	97
183	CUGGAUUCUUCCCCAGUGG	11	183	CUGGAUUCUUCCCCAGUGG	11	201	CCACUGGGAAAGAAUCCAG	98
201	GCUUJGCACUGAAACAGUGA	12	201	GCUUJGCACUGAAACAGUGA	12	219	UCACUGUUUCAGUGCAAGC	99
219	ACUUUJACUGAAGGGUGG	13	219	ACUUUJACUGAAGGGUGG	13	237	CCACACUUCUAGUAAAAGU	100
237	GAAGGAAAGGAAGGGGUU	14	237	GAAGGAAAGGAAGGGGUU	14	255	AACCCCAUUCUUCUUCUUC	101
255	UCCUUCUACUACUCCUUA	15	255	UCCUUCUACUACUCCUUA	15	273	UAAAGGAGUAGUAGAAGGA	102
273	AAGACUGAGCAUUUGAUAA	16	273	AAGACUGAGCAUUUGAUAA	16	291	UUAUCAAUUJGCUAGCUUU	103
291	ACUCUJGGGUCCUCUJUG	17	291	ACUCUJGGGUCCUCUJUG	17	309	CAAAGGAGCCACAGAGU	104
309	GUUUUJACCAUUGUUGGAA	18	309	GUUUUJACCAUUGUUGGAA	18	327	UCCCAACAAUGGUAAAAC	105
327	AACUCCGUUGGUUUUUU	19	327	AACUCCGUUGGUUUUUU	19	345	AAAAAGACAACGGAGGU	106
345	UCCACAUGGAGGAAAGA	20	345	UCCACAUGGAGGAAAGA	20	363	UCUUUCUCCUCAUGUGGA	107
363	AAGAAGUCAAGAAUGACCU	21	363	AAGAAGUCAAGAAUGACCU	21	381	AGGUCAUUCUUGACUUCU	108
381	UUCUJUUGACUJACUGGG	22	381	UUCUJUUGACUJACUGGG	22	399	CCAGCUGAGUACAAAGAA	109
399	GCCAUACAGAUUUCUUC	23	399	GCCAUACAGAUUUCUUC	23	417	UGAAAGAAUCUGUGAUGGG	110
417	ACAGGACUGGUCAACAUUC	24	417	ACAGGACUGGUCAACAUUC	24	435	AGAUGUJGACCAGUCCUGU	111
435	UUGACAGAUUAAAUGGC	25	435	UUGACAGAUUAAAUGGC	25	453	GCCAAUJAAUACUGUCAA	112
453	CGAUUCACUGGAGACUUCA	26	453	CGAUUCACUGGAGACUUCA	26	471	UGAAGUCUCCAGUGAAUCG	113
471	ACGGCACCUAGCCUGGUU	27	471	ACGGCACCUAGCCUGGUU	27	489	AAACCCAGGUAGGUCCGU	114
489	UGCCGAGGUUGGUCCGUUU	28	489	UGCCGAGGUUGGUCCGUUU	28	507	AAUAGGGGACCCACUCGGCA	115
507	UUGCAGGUUGGUUGGUCCU	29	507	UUGCAGGUUGGUUGGUCCU	29	525	AGAGCAGGACACAACCUGCAA	116
525	UACGCCUACUACGUCC	30	525	UACGCCUACUACGUCC	30	543	GGACGUAGGUAGGGCGUA	117
543	CUGGUGUCCCCUAGCAUAG	31	543	CUGGUGUCCCCUAGCAUAG	31	561	CUGGUGUAGGGACACCAAG	118

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID	Seq ID
561	GACAGAUACCAUGCCAUCG	32	561	GACAGAUACCAUGCCAUCG	32	579	CGAUGGCAUGGUAUCUGUC	119	
579	GUCUACCCCAUGGAAGUUC	33	579	GUCCACCCCAUGGAAGUUC	33	597	GGAAACLUCAUGGGGUAGAC	120	
597	CUUCAAGGAGAAAAGCAAG	34	597	CUUCAAGGAGAAAAGCAAG	34	615	CUUGGUUUUCUCCUUGAAG	121	
615	GCCAGGGGUCCUCAUUGUGA	35	615	GCCAGGGGUCCUCAUUGUGA	35	633	UCACAAUGGACCCUGGCC	122	
633	AUCGCCUGGAGCCUGUCU	36	633	AUCGCCUGGAGCCUGUCU	36	651	AAGACAGGCCUCCAGGCCAU	123	
651	UUUCUGUUCUCCAUUCCCCA	37	651	UUUCUGUUCUCCAUUCCCCA	37	669	UGGGAAUGGAGAACAGAAA	124	
669	ACCCUGAUCAUAUUUGGA	38	669	ACCCUGAUCAUAUUUGGA	38	687	UCCCCAAUUAUGAUCAAGGGU	125	
687	AAGAGGACACUGGUCCAACG	39	687	AAGAGGACACUGGUCCAACG	39	705	CGUUGGACAGUGUCCUCUU	126	
705	GGUGAAGUGCAGUGCUGGG	40	705	GGUGAAGUGCAGUGCUGGG	40	723	CCCAAGCACUGGCACUUCACC	127	
723	GCCCUGGGCCUGACGACU	41	723	GCCCUGGGCCUGACGACU	41	741	AGUCGUICAGGCCACAGGGC	128	
741	UCCUACUGGACCCAUACAU	42	741	UCCUACUGGACCCAUACAU	42	759	UGUAUAGGGGUCCAGUAGGA	129	
759	AUGACCAUCGUGGCCUUC	43	759	AUGACCAUCGUGGCCUUC	43	777	GGAAAGGCCACGGAUGGUCAU	130	
777	CUGGUGUACUCAUCCUC	44	777	CUGGUGUACUCAUCCUC	44	795	GAGGGGAUGGAAGUACACCAG	131	
795	CUGACAAUCAUCAGCAUC	45	795	CUGACAAUCAUCAGCAUC	45	813	UGAUGCUGAUGAUUGUCAG	132	
813	AUGUAUAGGCAUJUGUAUCC	46	813	AUGUAUAGGCAUJUGUAUCC	46	831	GGAUACACAAUGCCAUACAU	133	
831	CGAACUAUUUGGAUAAA	47	831	CGAACUAUUUGGAUAAA	47	849	UUUUAAUCCAAAUAJGUUCG	134	
849	AGCAAAACCUACGAAACAG	48	849	AGCAAAACCUACGAAACAG	48	867	CUGUUUUGGUAGGUUUGCU	135	
867	GUGAUUUCCAACUGGUCA	49	867	GUGAUUUCCAACUGGUCA	49	885	CUGAGCAGUGGGAAUACAG	136	
885	GAUGGGAAAACUGGAGCA	50	885	GAUGGGAAAACUGGAGCA	50	903	UGCUGCACAGGUUCCCAUC	137	
903	AGCUUAACCGAGGACUCA	51	903	AGCUUAACCGAGGACUCA	51	921	UGAGGUCCUCGGUUAUGCU	138	
921	AUCUCAAAGGCAAAAUCA	52	921	AUCUCAAAGGCAAAAUCA	52	939	UGAUUUUUGCCUUJUGAGAU	139	
939	AAGGCCUAUCAAGUAUAGCA	53	939	AAGGCCUAUCAAGUAUAGCA	53	957	UGCUUAUACUUGAUAGCCUU	140	
957	AUCAUCAUCAUUCUJGCCU	54	957	AUCAUCAUCAUUCUJGCCU	54	975	AGGCAAGAAUGAUGAUGAU	141	
975	UUCAUUCUGCUGUJGGAGUC	55	975	UUCAUUCUGCUGUJGGAGUC	55	993	GACUCCAACAGCAGAUGAA	142	
993	CCAUACUUCUCCUUUUGACA	56	993	CCAUACUUCUCCUUUUGACA	56	1011	UGCUAAACAGGAAGUAGUAG	143	
1011	AUUUUGGACAUUUJCAAC	57	1011	AUUUUGGACAUUUJCAAC	57	1029	GGUUGAAAUGGUCCAAAUAU	144	
1029	CUCCUCCAGACACCCAGG	58	1029	CUCCUCCAGACACCCAGG	58	1047	CCUGGGUGUGUGGAAGGGAG	145	
1047	GAGCGUUUCUUAUGCCUCUG	59	1047	GAGCGUUUCUUAUGCCUCUG	59	1065	CAGAGGCAUAGAAACGCU	146	
1065	GUGAUCAUUCAGAACCCUG	60	1065	GUGAUCAUUCAGAACCCUG	60	1083	GCAGGGUUCUGUAUGAUACAC	147	
1083	CCAGCAUUGAAUAGUGCCA	61	1083	CCAGCAUUGAAUAGUGCCA	61	1101	UGGCACAUUUCAUUGCUGG	148	
1101	AUCAACCCCCUCAUCUACU	62	1101	AUCAACCCCCUCAUCUACU	62	1119	AGUAGAUGAGGGGUUGAU	149	
1119	UGUGUCUUUCAGCAGCUCCA	63	1119	UGUGUCUUUCAGCAGCUCCA	63	1137	UGGAGCUGGUAGGACACAC	150	
1137	AUCUCUUUCCCCUCGCAGGG	64	1137	AUCUCUUUCCCCUCGCAGGG	64	1155	CCCUGCAGGGAAAAGAGAU	151	

Pos	Sq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
1155	GUCAUCCGUUCCGUCAGC	175	1155	GUCAUCCGUUCCGUCAGC	175	1173	GCUGACGGAGACGGGAUGAC	189
1173	CUCAGGAGGCUUGGCUAA	176	1173	CUCAGGAGGCUUGGCUAA	176	1191	UUAGCGCAGGCCUCUGGAG	190
1191	AUGCUCUGCCUCAACGAG	177	1191	AUGCUCUGCCUCAACGAG	177	1209	CUCGUUGAGGGCAGAGCAU	191
1209	GAGAACUGGAAGGGUACUU	178	1209	GAGAACUGGAAGGGUACUU	178	1227	AAGUACCCUUCAGUUCUC	192
1227	UGGCCAGGUGUACCUUCU	179	1227	UGGCCAGGUGUACCUUCU	179	1245	AGGAAGGUACACGUUGCCA	193
1245	UGGGCUCUUCAGGUGAC	180	1245	UGGGCUCUUCAGGUGAC	180	1263	GUACACCUUGGAAGAGGCCA	194
1263	CAGCUCUCACCCUGUGCUG	181	1263	CAGCUCUCACCCUGUGCUG	181	1281	CAGCACAGGGUGAGAGCUG	195
1281	GCAGGGGGCCUGUGCCUG	182	1281	GCAGGGGGCCUGUGCCUG	182	1299	CAGGCACAGGGCACCCUGC	196
1299	GGGCCACUUCUCACUGCU	183	1299	GGGCCACUUCUCACUGCU	183	1317	AGCAGUGAGAUGUGGCCACC	197
1317	UUACCCAGGGCACAAGGACA	184	1317	UUACCCAGGGCACAAGGACA	184	1335	UGUCCUUGGGCCUGGUAA	198
1335	ACCAAGGGUUCCCCAAAUG	185	1335	ACCAAGGGUUCCCCAAAUG	185	1353	CAUJJUGGAACACACUGGU	199
1353	GGGUACAGCAGGAUGGCC	186	1353	GGGUACAGCAGGAUGGCC	186	1371	GGCCCAUCCUGCUGGACCC	200
1371	CUGCAUCAGAUUCACCAGG	187	1371	CUGCAUCAGAUUCACCAGG	187	1389	CCUGGGUAAUCUGAUGCAG	201
1389	GGAGGGCUAUAGAAGGCA	188	1389	GGAGGGCUAUAGAAGGCA	188	1407	UGCCCUUCUUAUGGCCCUCC	202

## AAA-2

Pos	Seq	Seq ID	Up ID	Up Pos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3	UGAUGGGGAAGGAGAAUG	203	3	UGAUGGGGAAGGAGAAUG	203	21	CAUUCUCCUCCACCAUCAC	222	
21	GAGUCUCUGAUGCCTUUGG	204	21	GAGUCUCUGAUGCCTUUGG	204	39	CCAAAGGCAUCAGAGACUC	223	
39	GACUUGAUGCUGGAAAGAC	205	39	GACUUGAUGCUGGAAAGAC	205	57	GUCCUUCAGCAUCAGAGUC	224	
57	CUUAAGACUJJGGGGACU	206	57	CUUAAGACUJJGGGGACU	206	75	AGUCCCCCAAAGGUUUAAG	225	
75	UACUGGAAAGGAGUGACUU	207	75	UACUGGAAAGGAGUGACUU	207	93	AAGUCACUCCUJJUCAGUA	226	
93	UCUCCCAGAUUUUGUAU	208	93	UCUCCCAGAUUUUGUAU	208	111	AUACAAAAAUICUGGGGAGA	227	
111	UACCUUGACUCUGUUCAGC	209	111	UACCUUGACUCUGUUCAGC	209	129	GCUGAAAACAGAGUCAGGUA	228	
129	CAUCCGGCUUCCAAAGAAU	210	129	CAUCCGGCUUCCAAAGAAU	210	147	AUUCUUUGGGAAAGCGGAUG	229	
147	UGCAUGUGAAGCAGGGC	211	147	UGCAUGUGAAGCAGGGC	211	165	GCUCUCUGCUUCACACUGCA	230	
165	CUUAGUGAGAAAGAACGC	212	165	CUUAGUGAGAAAGAACGC	212	183	GCGUUUUCUUCACAUAAAG	231	
183	CAGGGAGACAGUICAGUCA	213	183	CAGGGAGACAGUICAGUCA	213	201	UGACUGAAACUGUCUCCUG	232	
201	ACUGCAAAUCUUCUAGGCCA	214	201	ACUGCAAAUCUUCUAGGCCA	214	219	UGGGCAUGAAAGAUJGCAGU	233	
219	AUCAGUUUCUJUGUGAGAG	215	219	AUCAGUUUCUJUGUGAGAG	215	237	CUUCUCACAAAGAAACUGAU	234	
237	GAAAACAAGUUGAUUACA	216	237	GAAAACAAGUUGAUUACA	216	255	UGUAUAUCCACUUGUUUUC	235	
255	ACUGGUCCAAGCAGCAUGU	217	255	ACUGGUCCAAGCAGCAUGU	217	273	ACAUGCUGCUUGGAACAGU	236	
273	UGUUGAAAAGAUUUUGUCUU	218	273	UGUUGAAAAGAUUUUGUCUU	218	291	AAGACAAAUCUUUUCAACA	237	
291	UUUCCCCAUUAUGGUUCU	219	291	UUUCCCCAUUAUGGUUCU	219	309	AGACCAUUAAAUGGGAAA	238	
309	UUGGUACCUUUCUCAAAA	220	309	UUGGUACCUUUCUCAAAA	220	327	UUIUUJUGAGAAAAGGUACCAA	239	
321	UCAAAAAUUGACCAUUAU	221	321	UCAAAAAUUGACCAUUAU	221	339	AUUAUJGGUCAUUUJUGA	240	

## AAA1-3

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3	UAGGACUCAGAAAUAUAGA	241	3	UAGGACUCAGAAAUAUAGA	241	21	UCUAUAUUUCUGAGGUCCUA	281
21	AUGUUAGUAAGGCAAACA	242	21	AUGUUAGUAAGGCAAACA	242	39	UGUUGGCUCUUAACAU	282
39	AGACAUAAACAGAAACACA	243	39	AGACAUAAACAGAAACACA	243	57	UGUGUUAUCUGUAUGUUCU	283
57	AUACAAAGUGCUACCACA	244	57	AUACAAAGUGCUACCACA	244	75	UGUGUAGGCAUUUGUAU	284
75	AUGCUAACCAUCGUGCA	245	75	AUGCUAACCAUCGUGCA	245	93	CGUGAGCAGGUAGGUAGCAU	285
93	GGCACUUUCUAUAGAAGAA	246	93	GGCACUUUCUAUAGAAGAA	246	111	UUCUUCUUAUAGAAGUGCC	286
111	ACUAAUUUAUCAUCACCA	247	111	ACUAAUUUAUCAUCACCA	247	129	UGUGUAGUUAUAAUAGU	287
129	AUAACCCUAUGGGGUAGAU	248	129	AUAACCCUAUGGGGUAGAU	248	147	AUCUACCCCACAUAGGGGUUAU	288
147	UGAUUUUUUACAAACCUCC	249	147	UGAUUUUUUACAAACCUCC	249	165	GGAGGUUGUAAAAAAUACA	289
165	CAUUUACAGAUGAAGAAA	250	165	CAUUUACAGAUGAAGAAA	250	183	UUUCUUCAUUCGUAAAUG	290
183	ACUGGAAGCAUAGACCUGCU	251	183	ACUGGAAGCAUAGACCUGCU	251	201	AGCAGGCGCUAUGCUCUUCAGU	291
201	UUAUGUGAGAAACGCA	252	201	UUAUGUGAGAAACGCA	252	219	UGCGUUUCUUCUACAUAA	292
219	AGGGAGACAGUUCAGUCAC	253	219	AGGGAGACAGUUCAGUCAC	253	237	GUGACIUGAACIUGUCUCCU	293
237	CUGGAAUCUUCAGGCCAU	254	237	CUGGAAUCUUCAGGCCAU	254	255	AUGGGCAUGAAGAUUGCAG	294
255	UCAGUUUCUUGAGAAGA	255	255	UCAGUUUCUUGAGAAGA	255	273	UCUUCUCAAGAACUGA	295
273	AAAACAAGAAAACAAGGAC	256	273	AAAACAAGAAAACAAGGAC	256	291	GUCCUUGUUUCUUGUUUU	296
291	CUGAAAUCACAGGAAG	257	291	CUGAAAUCACAGGAAG	257	309	CUUCCUGUGGGAUUUUCAG	297
309	GGUGGCAGUGACUCCACA	258	309	GGUGGCAGUGACUCCACA	258	327	UGGGAGGUUCACUGCCACC	298
327	AGACGGGACCUUGACGCCUC	259	327	AGACGGGACCUUGACGCCUC	259	345	GAGGGGUCCAGGUCCGUCCU	299
345	CAACACUCCUGGCCUUAAC	260	345	CAACACUCCUGGCCUUAAC	260	363	GGUAAGGCCAGGAGGUUG	300
363	CUCCCUUGCUAACGUCUC	261	363	CUCCCUUGCUAACGUCUC	261	381	GAGACGUUCAGCAAGGGAG	301
381	CAAGUUUCUUCUGGUUCAG	262	381	CAAGUUUCUUCUGGUUCAG	262	399	CUGAACGGCAGAAACUUG	302
399	GGACUGGCAACGCCUGCU	263	399	GGACUGGCAACGCCUGCU	263	417	AAGCAGGGGUUGCCAGUCC	303
417	UCCUCCUCUGAGCUGUCAA	264	417	UCCUCCUCUGAGCUGUCAA	264	435	UJGACAGCUUCAGGGAGGA	304
435	AGUAGGAAGGUCCGGGUGC	265	435	AGUAGGAAGGUCCGGGUGC	265	453	GCAGCCGGGACUCCUACU	305
453	CUCUGCUAGAAAAGAAGU	266	453	CUCUGCUAGAAAAGAAGU	266	471	ACUUUCUUCUUCAGCAGAG	306
471	UCAUGUGCAGGAGCACUGA	267	471	UCAUGUGCAGGAGCACUGA	267	489	UCAGUGGUCCUCGACAU	307
489	AGGCAUCCAGGUGUGACA	268	489	AGGCAUCCAGGUGUGACA	268	507	UGUCACACCUGGGAUGGCC	308
507	ACUCIUCUCCACCUAGAGCAU	269	507	ACUCIUCUCCACCUAGAGCAU	269	525	AUGCUUCIAGGUGGAAAGAGU	309
525	UUCGGUCUCUCAUCCUCUG	270	525	UUCGGUCUCUCAUCCUCUG	270	543	CAGAGGAUGAGAGACGGAA	310
543	GCCAUUGUGACGGCUGGGCU	271	543	GCCAUUGUGACGGCUGGGCU	271	561	AAGCCCAGCGGUACAUAGGC	311

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
561	UCUUUACAAAUAUCC	272	561	UCUUUACAAAUAUCC	272	579	GGGAUJAAUJUGUAAAAGA	312
579	CAAGUGCAAGACAUUAUU	273	579	CAAGUGCAAGACAUUAUU	273	597	AAUAAUAGUCUJUGCACUUG	313
597	UUCUUCUGUACCUAUGAC	274	597	UUCUUCUGUACCUAUGAC	274	615	GUCAUJAGGUACAGAAAGAA	314
615	CCUGAGCAAUCUUCUCUG	275	615	CCUGAGCAAUCUUCUCUG	275	633	CAGAGAAGGGAUJUGCUCAGG	315
633	GCUGAACCCUGGUAGUGUCA	276	633	GCUGAACCCUGGUAGUGUCA	276	651	UGACACUACCCAGGUUCAGC	316
651	AUCUUUAGAAUGGAAGACA	277	651	AUCUUUAGAAUGGAAGACA	277	669	UGUCUUCACUUCUAAAAGAU	317
669	ACAAUUUACACACAGGUCAU	278	669	ACAAUUUACACACAGGUCAU	278	687	AUGACCAUGUGGUAAAUGU	318
687	UUUCUUCAUUAUACGUUG	279	687	UUUCUUCAUUAUACGUUG	279	705	CAACGAAUAAAUGAAGAAA	319
690	CUUCAUUUAUCCGUUGUUA	280	690	CUUCAUUUAUCCGUUGUUA	280	708	UAACAAACGAUUAUJUGAAG	320

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Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3	UAGGACUCAGAAAUUAGA	241	3	UAGGACUCAGAAAUUAGA	241	21	UCUAUAUUUCAGAGGUCAA	281
21	AUGUUAUGUAAGGCAAACA	242	21	AUGUUAUGUAAGGCAAACA	242	39	UGUUGUCUUAACUACAU	282
39	AGACAUAAACAGAUAAACACA	243	39	AGACAUAAACAGAUAAACACA	243	57	UGUGUUAUCUGUAUGUCU	283
57	AUACAAAUGGGCUACCCACA	244	57	AUACAAAUGGGCUACCCACA	244	75	UGGGUAGGGCACUUGUAU	284
75	AUGCUAAACCACIUGCUGCAG	245	75	AUGCUAAACCACIUGCUGCAG	245	93	CUGCAGCAGGGUAGCAU	285
93	GGCACUUUCUUAAGAAGAA	246	93	GGCACUUUCUUAAGAAGAA	246	111	UUCUUCUUAAGAAAGUGCC	286
111	ACUAAUAAAUCAUACCCA	247	111	ACUAAUAAAUCAUACCCA	247	129	UGGUGAUGAUUAAAUAUGU	287
129	AUAACCCUAUGGGGUAGAU	248	129	AUAACCCUAUGGGGUAGAU	248	147	AUCUACCCCAUAGGGGUUAU	288
147	UGAUUUUUUACAAACCUCC	249	147	UGAUUUUUUACAAACCUCC	249	165	GGAGGIIUJGJAAAAAUUACA	289
165	CAUUUACAGAUGAAGAAA	250	165	CAUUUACAGAUGAAGAAA	250	183	UUUCUJCAUCUGUAAAUG	290
183	ACUGAAAGCAUAGACCUGU	251	183	ACUGAAAGCAUAGACCUGU	251	201	AGCAGGGCUCUAUGGUUCAGU	291
201	UUAUUGGAGAAAGAAACGCA	252	201	UUAUUGGAGAAAGAAACGCA	252	219	UGCGUUUCUUCUACAUAA	292
219	AGGGAGACAGUUCAGUCAC	253	219	AGGGAGACAGUUCAGUCAC	253	237	GUGACUGAACUGUCUCCU	293
237	CUGCAAUCUCAUGCCCCAU	254	237	CUGCAAUCUCAUGCCCCAU	254	255	AUGGGCAUGAAGAUUGCAG	294
255	UCAGUUUUUJGUGAGAAGA	255	255	UCAGUUUUUJGUGAGAAGA	255	273	UCUUUCUCAAGAAACUGA	295
273	AAAACAGACUGGCAACGC	273	AAAACAGACUGGCAACGC	273	291	GCGUUGGCCAGUCIJGUUJU	359	
291	CCUGCUUCCUCUCUGAGC	291	291	CCUGCUUCCUCUCUGAGC	291	309	GCUCAAGGGAGGAAGCAGG	360
309	CUGUCAAGUAGGAAGGUCCG	309	309	CUGUCAAGUAGGAAGGUCCG	309	327	CGGACLUCCUACIJUGACAG	361
327	GGGCUJGCUJGCUJGAAAG	327	GGGCUJGCUJGCUJGAAAG	327	345	CUUUUAGGAGGAGGAGGCC	362	
345	GAGAAAGCUAUGUGGAGGAG	325	345	GAGAAAGCUAUGUGGAGGAG	325	363	CUCCUGCACAUAGACUUCUC	363
363	GCACUGGGCAUCCAGGU	326	363	GCACUGGGCAUCCAGGU	326	381	ACCUGGGGAUGCCUJAGUGC	364
381	UGUGACACUCUJCCACCJA	327	381	UGUGACACUCUJCCACCJA	327	399	UAGGGUGGAAGAGJUGUCACA	365
399	AGAGCAUUCGJUCUCAU	328	399	AGAGCAUUCGJUCUCAU	328	417	AUGAGGAGCAGGAAUGCUCU	366
417	UCCUCUGCCAUAGUAGCAA	329	417	UCCUCUGCCAUAGUAGCAA	329	435	UUUGCUCACAUGGAGAGGA	367
435	ACUGCUAUGCAUCUUCAG	330	435	ACUGCUAUGCAUCUUCAG	330	453	CUGAAGGAUGCAUAGCAGU	368
453	GCUGCAAGGGAUJGAAUGC	331	453	GCUGCAAGGGAUJGAAUGC	331	471	GCAUUCAAUCCUJUGCAGC	369
471	CUAUCAACAACAUACAAG	332	471	CUAUCAACAACAUACAAG	332	489	CUUUGUAUGGUUGUJUGAUAG	370
489	GUGGAGAAGCAGAUGCUUC	333	489	GUGGAGAAGCAGAUGCUUC	333	507	GAAGGCAUCUGCUUCUCCAC	371
507	CCCUAGCJUGAGGCCUCAGGC	334	507	CCCUAGCJUGAGGCCUCAGGC	334	525	GCCUGAGGGCUCAGCJUAGGG	372
525	CUUUUUGGAGAAUUGCUA	335	525	CUUUUUGGAGAAUUGCUA	335	543	UAGCAAUCCAUAAAAG	373
543	ACAACUJGGGGCAUGGCCUG	336	543	ACAACUJGGGGCAUGGCCUG	336	561	CAGGCAUGCCAUAGGUUGU	374

Pos	Seq	Seq ID	UpPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
561	GCUCUAAAAGAAUACUC	337	561	GCUCUAAAAGAAUACUC	337	579	GAGUAUUUUCUUUAGGAGC	375
579	CAGGAUUGUCUCAUAAAG	338	579	CAGGAUUGUCUCAUAAAG	338	597	CUUUAUGAGACAAUUCUG	376
597	GUCCUCACCUACUGGCAA	339	597	GUCCUCACCUACUGGCAA	339	615	UUUGCCAGUAGGUAGGAC	377
615	AAACAAGAUGUCUACUC	340	615	AAACAAGAUGUCUACUC	340	633	GGAGUAGAACAUUUGUUU	378
633	CCAGGUAGACUUUUGAAG	341	633	CCAGGUAGACUUUUGAAG	341	651	CUUAGAAAAGUCAACCUGG	379
651	GCCCCAAGAUGUAGUCA	342	651	GCCCCAAGAUGUAGUCA	342	669	UGACUCAACAUUCUJGGGC	380
669	AGCCAUUCUCCAGGAUCU	343	669	AGCCAUUCUCCAGGAUCU	343	687	AGAUCCUUGGAGAAUGGU	381
687	UCGAUUUCUUUUAUGGA	344	687	UCGAUUUCUUUUAUGGA	344	705	UCCAUAAAAGGAAAUCGA	382
705	AAAAAUACAUAAAACACCA	345	705	AAAAAUACAUAAAACACCA	345	723	UGGUGUUUAUGGUAAUUU	383
723	AAAUAAGCCUCGCUGUC	346	723	AAAUAAGCCUCGCUGUC	346	741	GACAGCGAGGCUUAUAAA	384
741	CCCACAUUGCGUAUUGGGGA	347	741	CCCACAUUGCGUAUUGGGGA	347	759	UCCCCAAUACGCAGUGGG	385
759	ACAAGAUGAAACCUGCUUC	348	759	ACAAGAUGAAACCUGCUUC	348	777	GAAGCAGGUUUCAUUUGU	386
777	CCAGGCCUACUJJGGCAGCA	349	777	CCAGGCCUACUJJGGCAGCA	349	795	UGCUGCCAAAGUAGCCUGG	387
795	AGAACUGAAAAGGCCUUUU	350	795	AGAACUGAAAAGGCCUUUU	350	813	AAAAGCCUUUUUCAGUUCU	388
813	UUUUCAGAUUAUGAUUU	351	813	UUUUCAGAUUAUGAUUU	351	831	AAUCAUUAUCUGGAAAA	389
831	UCUCAUCGACAGGGUUGCA	352	831	UCUCAUCGACAGGGUUGCA	352	849	UGCAACCCUGUGGAUGAGA	390
849	ACAGGCCUCUUUUUGUUUC	353	849	ACAGGCCUCUUUUUGUUUC	353	867	GAACAAUAAAGGGCUGU	391
867	CGUJGUAAAUGACACCCUUG	354	867	CGUJGUAAAUGACACCCUUG	354	885	CAAGGGUGUCAUUUACACG	392
885	GGAUUCUGAACAUACACAC	355	885	GGAUUCUGAACAUACACAC	355	903	GUGUGUUUGUUCAGAUCC	393
903	CCAGGACAAUUGUGGCAA	356	903	CCAGGACAAUUGUGGCAA	356	921	UUGCACACAAUUGGUCCUGG	394
921	ACAGGUUCUACAAACUGAU	357	921	ACAGGUUCUACAAACUGAU	357	939	UAUCAGUUUGUAGAACUGU	395
929	ACAAACUGAUUUUCUAAU	358	929	ACAAACUGAUUUUCUAAU	358	947	AUUAGAAAUAUCAGUUUGU	396

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Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3	UAGGACUCAGAAUUAUAGA	241	3	UAGGACUCAGAAUUAUAGA	241	21	UCUAUAUUUUCAGAGGUCCUA	281
21	AUGUUAGUAAGGCAAAACA	242	21	AUGUUAGUAAGGCAAAACA	242	39	UGUUUUCUUCUACUAACAU	282
39	AGACAUAAACAGAUAAACACA	243	39	AGACAUAAACAGAUAAACACA	243	57	UGUGUUAUCUGUAUGUCU	283
57	AUACAAAAGUGCCUACCACA	244	57	AUACAAAAGUGCCUACCACA	244	75	UGUGUUAUCUGUAUGUUAU	284
75	AUGCUAAACCACUGCGCAG	245	75	AUGCUAAACCACUGCGCAG	245	93	CUGCAGCAGUGGUAGCAU	285
93	GGCACUUCUCAUAGAAAGAA	246	93	GGCACUUCUCAUAGAAAGAA	246	111	UUCUUCUUAUAGAAAGUCC	286
111	ACUAUUAUUAUCAUCACCA	247	111	ACUAUUAUUAUCAUCACCA	247	129	UGGUGAUGUUAUUUUAGU	287
129	AUAACCCUAUGGGGUAGAU	248	129	AUAACCCUAUGGGGUAGAU	248	147	AUCUACCCCUCUAGGGGUUAU	288
147	UGAUUUUUUUAACCUC	249	147	UGAUUUUUUUAACCUC	249	165	GGAGGUUGUAAAAAUUAUCA	289
165	CAUUUACAGAUGAAGAAA	250	165	CAUUUACAGAUGAAGAAA	250	183	UUUCUCAUCUGUAAAUG	290
183	ACUGAAGCAUAGACCUGCU	251	183	ACUGAAGCAUAGACCUGCU	251	201	AGCAGGCUUAUGGUUCAGU	291
201	UUAUGUGAGAAAGGCA	252	201	UUAUGUGAGAAAGGCA	252	219	UGCGUUUCUUCUACAUAAA	292
219	AGGGAGACAGUUCAGUCAC	253	219	AGGGAGACAGUUCAGUCAC	253	237	GUGACUGAACUGUCUCCU	293
237	CUGCAAAUCUCAUGCCAU	254	237	CUGCAAAUCUCAUGCCAU	254	255	AUGGGCAUGAAGAUUGCAG	294
255	UCAGUUUUUCUUGUGAGAAGA	255	255	UCAGUUUUUCUUGUGAGAAGA	255	273	UCUUCUCAAGAAACUGA	295
273	AAAACAAAAGCAAAUCGUA	297	273	AAAACAAAAGCAAAUCGUA	297	291	UAGCAGUUUUCUUGUUUUU	426
291	AUGCAUCUUCAGCGUCAA	298	291	AUGCAUCUUCAGCGUCAA	298	309	UUGCAGCUGAAGGAUGCAU	427
309	AGGAUUGAAUGCUAUCAA	399	309	AGGAUUGAAUGCUAUCAA	399	327	UUGAUAGCAUUCUAAUCCU	428
327	ACAACCAUACAAGUGGAGA	400	327	ACAACCAUACAAGUGGAGA	400	345	UCUCCACUUCUAGGGUUGU	429
345	AAGCAGAUGCUCUCCUAGC	401	345	AAGCAGAUGCUCUCCUAGC	401	363	GCUAGGGAAAGCAUCUGCUU	430
363	CUGAGCCUCAGGCUUUUG	402	363	CUGAGCCUCAGGCUUUUG	402	381	CAAAAGCCUGAGGCUCAG	431
381	GAUGGAAUUGCUACACUJ	403	381	GAUGGAAUUGCUACACUJ	403	399	AAGUUGUAGCAAUUCCAUC	432
399	UGGUGCAUGCUCUGCUCCUA	404	399	UGGUGCAUGCUCUGCUCCUA	404	417	UAGGAGCAGGCAUGCACCA	433
417	AAAAGAAAUAUCUCAGGAU	405	417	AAAAGAAAUAUCUCAGGAU	405	435	AUUCUUCAGUAAUUCUUUU	434
435	UUGUCUCUAAAGGUCCUCA	406	435	UUGUCUCUAAAGGUCCUCA	406	453	UGAGGACUUUAUGAGACAA	435
453	ACCUACUGGCAAAACAAG	407	453	ACCUACUGGCAAAACAAG	407	471	CUUGUUUUUGCCAGUAGGU	436
471	GAUGUUUCUACUCCCGGCU	408	471	GAUGUUUCUACUCCCGGCU	408	489	AACCUGGGAGUAGAACAU	437
489	UGACUUUUUCAGCCCCAA	409	489	UGACUUUUUCAGCCCCAA	409	507	UUGGGCUUUGAAAAAGUCA	438
507	AGAUGUUGAGUAGCCAUU	410	507	AGAUGUUGAGUAGCCAUU	410	525	AAUGGCUGACUCAACAUU	439
525	UCUCCAAGGAUCUCGAUU	411	525	UCUCCAAGGAUCUCGAUU	411	543	AAAUCGAGAUCUCUUGGAGA	440
543	UCCUUUUUAUGGAAAAAAA	412	543	UCCUUUUUAUGGAAAAAAA	412	561	UUUUUUUCUUAUAAAAGGA	441

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID	Seq ID
561	ACAUAAAACACCAAAUA	413	561	ACAUAAAACACCAAAUA	413	579	UAUAAAUGGUGUUAAUGU	442	
579	AAGCUCUCGCUGUCCCCAU	414	579	AAGCUCUCGCUGUCCCCAU	414	597	AUGUGGGACAGCGAGGCCU	443	
597	UGCGUAUUGGGACAAGAU	415	597	UGCGUAUUGGGACAAGAU	415	615	AUCUUGUCCCCAUACGCA	444	
615	UGAAACCUGCUUCCAGGU	416	615	UGAAACCUGCUUCCAGGU	416	633	AGCCUUGGAAGCAGGUUCA	445	
633	UACUUUGGCAGCAGAACU	417	633	UACUUUGGCAGCAGAACU	417	651	CAGUUCUGCUGCCAAAGUA	446	
651	GAAAAAGGCCUUUUUCCA	418	651	GAAAAAGGCCUUUUUCCA	418	669	UGGAAAAAAGCCUUUUUC	447	
669	AGAUUAUGAUUUUCAUUC	419	669	AGAUUAUGAUUUUCAUUC	419	687	GAUGAGAAAUCAUUAUUCU	448	
687	CGACAGGGGUUGCACAGCCC	420	687	CGACAGGGGUUGCACAGCCC	420	705	GGCGUGGCAACCCUGUCG	449	
705	CUCUJJUAUUGCUGGUAA	421	705	CUCUJJUAUUGCUGGUAA	421	723	UUAACACGAAACAUAAAGAG	450	
723	AAUGACACCCUJGGGAUCUG	422	723	AAUGACACCCUJGGGAUCUG	422	741	CAGAUCCAAGGGGUUCAUU	451	
741	GAACAAUACACACCGGAC	423	741	GAACAAUACACACCGGAC	423	759	GUCCUGGUGGUAGUUGUUC	452	
759	CAAUUGUGGCAACAGUU	424	759	CAAUUGUGGCAACAGUU	424	777	GAACUJGUUGCACACAAUUG	453	
777	CUACAAACUGUAUUCUA	425	777	CUACAAACUGUAUUCUA	425	795	UAGAAAUAUCAGUUUGUAG	454	
779	ACAAACUGUAUUCUAU	358	779	ACAAACUGUAUUCUAU	358	797	AUUAGAAAUAUCAGUUUGU	396	

## AAA1-6

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID	Lower seq	Seq ID
3	UGAUGGUGGAAGGAGAAUG	203	3	UGAUGGUGGAAGGAGAAUG	203	21	CAUUCUCCUUCACCAUCA	222		
21	GAGUCUCUGAUGCUCUUUGG	204	21	GAGUCUCUGAUGCUCUUUGG	204	39	CCAAAGGGCAUCAGAGACUC	223		
39	GACUUGAUGCUGGAAAGAC	205	39	GACUUGAUGCUGGAAAGAC	205	57	GUUUUUCAGCAUCAAAGUC	224		
57	CUUAAGACUJUUGGGGACU	206	57	CUUAAGACUJUUGGGGACU	206	75	AGUCCCCAAAGUCUUAAG	225		
75	UACUGGAAAGCUUAUGUGA	455	75	UACUGGAAAGCUUAUGUGA	455	93	UCACAUAAAGCUUUCCAGUA	497		
93	AGAAGAAAGGAGGGAGAC	456	93	AGAAGAAAGGAGGGAGAC	456	111	GUUCUCCUGGGUUIUCUUCU	498		
111	CAGUUCAGUCACUGCAAUC	457	111	CAGUUCAGUCACUGCAAUC	457	129	GAUUGCAGUGACUGAACUG	499		
129	CUUCUAGGCCCAUCAGUUUC	458	129	CUUCUAGGCCCAUCAGUUUC	458	147	GAAACUJGAUGGGCAUGAAG	500		
147	CUUUGAGAAAGAAAAACAG	459	147	CUUUGAGAAAGAAAAACAG	459	165	CUUUGUUUCUUCUACAAAG	501		
165	GACUGGCAACGCCUGCUUC	460	165	GACUGGCAACGCCUGCUUC	460	183	GAAGCAGGGGUUGGCCAGUC	502		
183	CCUCUCUGAGCUGUCAAG	461	183	CCUCUCUGAGCUGUCAAG	461	201	CUUGACAGCUAGGAGGAG	503		
201	GUAGGAAGGUCCCCGUGCU	462	201	GUAGGAAGGUCCCCGUGCU	462	219	AGCAGGGGGACUUCCUAC	504		
219	UCUGCUAGAAAGGAAGUC	463	219	UCUGCUAGAAAGGAAGUC	463	237	GACUUCUUCUUCUAGCAGA	505		
237	CAUGUGCAGGAGCACUGAG	464	237	CAUGUGCAGGAGCACUGAG	464	255	CUCAGUGCUCCUGGCACAU	506		
255	GGCAUCCCAGGUGACAC	465	255	GGCAUCCCAGGUGACAC	465	273	GUGUCACACCUGGAUGGCC	507		
273	CUCUCCACCUAGGCAUJ	466	273	CUCUCCACCUAGGCAUJ	466	291	AAUGCUCUAGGUGGAAGAG	508		
291	UCCGUUCUCUACCCUCUGC	467	291	UCCGUUCUCUACCCUCUGC	467	309	GCAGAGGAUGGAGACGGGA	509		
309	CCAUGUAGCAAACUGCUAU	468	309	CCAUGUAGCAAACUGCUAU	468	327	AUAGCAGUUUGCACAUAGG	510		
327	UGCAUCCUUCAGCUGCAAG	469	327	UGCAUCCUUCAGCUGCAAG	469	345	CUUGCAUCUGAAGGAUGCA	511		
345	GGGAUUGAAUGCUUAAC	470	345	GGGAUUGAAUGCUUAAC	470	363	GUUGAUAGCAUCUAAUCCC	512		
363	CAACCAUACAAAGGGAGAA	471	363	CAACCAUACAAAGGGAGAA	471	381	UUCUCCACUUUGUAGGUUG	513		
381	AGCAGAUGCUUCCUAGCU	472	381	AGCAGAUGCUUCCUAGCU	472	399	AGCUAGGGAAAGCAUCUGCU	514		
399	UGAGGCCUCAGGCUUUUGA	473	399	UGAGGCCUCAGGCUUUUGA	473	417	UCAAAAAGGCCUGAGGCUCA	515		
417	AUGGAAUUGCUCACAUJG	474	417	AUGGAAUUGCUCACAUJG	474	435	CAAGUJGUAGCAAUUCCAU	516		
435	GGUGCAUGGCCUCUUA	475	435	GGUGCAUGGCCUCUUA	475	453	UJAGGGAGGGCAUGGCACC	517		
453	AAAGAAAUAUCUAGGAAUJ	476	453	AAAGAAAUAUCUAGGAAUJ	476	471	AAUUCUCAGUAUUCUUUU	518		
471	UGUCUCUAAGGUCCUCAC	477	471	UGUCUCUAAGGUCCUCAC	477	489	GUGAGGACUUUAUGAGACAA	519		
489	CCUACUGGCCAAAACAAGA	478	489	CCUACUGGCCAAAACAAGA	478	507	UCUUGUJUUUGCAGUAGG	520		
507	AUGUUCUACUCCAGGUJG	479	507	AUGUUCUACUCCAGGUJG	479	525	CAACCUGGGAGUAGAACAU	521		
525	GACUUUUUCAAGCCCCAAG	480	525	GACUUUUUCAAGCCCCAAG	480	543	CUUJGGGUUCAAGAAAAAGUC	522		
543	GAUGUJUGAGUCAGGCCAUUC	481	543	GAUGUJUGAGUCAGGCCAUUC	481	561	GAAUGGCCUGACUCAACAU	523		

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID	Seq ID
561	CUCCAAGGAUCUCGAUUC	482	561	CUCCAAGGAUCUCGAUUC	482	579	GAAAUCGAGAUCUCUUGGAG	524	
579	CCUUUUUAAUGGAAAAAAC	483	579	CCUUUUUAAUGGAAAAAAC	483	597	GUUAUUUUCCAUUAAAAGG	525	
597	CAUJAAACACCAAAUAAA	484	597	CAUJAAACACCAAAUAAA	484	615	UUAUUUUCCGGGUUAAAUG	526	
615	AGCCUCGCGUICGCCACAU	485	615	AGCCUCGCGUICGCCACAU	485	633	CAUGUGGGACAGCGAGGCCU	527	
633	GCGUAUJUGGGGACAAGAU	486	633	GCGUAUJUGGGGACAAGAU	486	651	CAUCUJUGUCCCCAUACGC	528	
651	GAAACCUGCUUCCAGGCJA	487	651	GAAACCUGCUUCCAGGCJA	487	669	UAGCCUGGAAGCAGGUUUC	529	
669	ACUUUGGCAGCAGAACUGA	488	669	ACUUUGGCAGCAGAACUGA	488	687	UCAGUUCUGGUCCCAAAGU	530	
687	AAAAAGGCUUUUUUCAG	489	687	AAAAAGGCUUUUUUCAG	489	705	CUGGAAAAAAAGCCUUUUU	531	
705	GAUUAUUGAUUUUCUCAUCG	490	705	GAUUAUUGAUUUUCUCAUCG	490	723	CGAUGAGAAAUCAUUAUC	532	
723	GACAGGGGUUGGCACAGGCCU	491	723	GACAGGGGUUGGCACAGGCCU	491	741	AGGGCUGUGCAACCCUGUC	533	
741	UCUUUAUUGUUUCUGGUAAA	492	741	UCUUUAUUGUUUCUGGUAAA	492	759	UUUACACGAACAAUAAAAGA	534	
759	AUGACACCCUUUGGAUCUGA	493	759	AUGACACCCUUUGGAUCUGA	493	777	UCAGAUCCAAGGGUGUCAU	535	
777	AACAAUACACACCAGGACA	494	777	AACAAUACACACCAGGACA	494	795	UGUCCUGGUUGGUUUGGUU	536	
795	AAUUGUGUGGCAACAGUUCU	495	795	AAUUGUGUGGCAACAGUUCU	495	813	AGAACUGUUGGCACACAAUU	537	
813	UACAAACUGAUUUUCUAA	496	813	UACAAACUGAUUUUCUAA	496	831	UUAGAAAUAUCAGUUUGUA	538	
814	ACAAACUGAUUUUCUAAU	358	814	ACAAACUGAUUUUCUAAU	358	832	AUUAGAAAUAUCAGUUUGU	396	

AAA1-7

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID	Seq ID
3	UAGGACUCAGAAAUUAGA	241	3	UAGGACUCAGAAAUUAGA	241	21	UCUUAUAAAUCUGAGGUCCUA	281	
21	AUGUUAGUAAGGCAAACA	242	21	AUGUUAGUAAGGCAAACA	242	39	UGUUUUCUCUUACUAAACAU	282	
39	AGACAUAAACAGAAUACACA	243	39	AGACAUAAACAGAAUACACA	243	57	UGUGUAUCGUUAUGUCU	283	
57	AUACAAAGUGGCUACCACA	244	57	AUACAAAGUGGCUACCACA	244	75	UGUGGUAGGCCACUUGUUAU	284	
75	AUGCUAAACCACUGCUGCA	245	75	AUGCUAAACCACUGCUGCA	245	93	CUGGAGCAGUGGUAGCAU	285	
93	GGCACUJJUUCUAUAGAAGAA	246	93	GGCACUJJUUCUAUAGAAGAA	246	111	UUCUUCUUAUAGAAAGGCC	286	
111	ACUAAUJUUAUCACCA	247	111	ACUAAUJUUAUCACCA	247	129	UGGUGAUGAUAAAUAUAGU	287	
129	AUAACCCUAUGGGGUAGAU	248	129	AUAACCCUAUGGGGUAGAU	248	147	AUCUACCCCACGGGUUAU	288	
147	UGAUUUUJUUAACCUUC	249	147	UGAUUUUJUUAACCUUC	249	165	GGAGGGUJGUAAAUAUCAUCA	289	
165	CAUUUACAGAUGAAGAAA	250	165	CAUUUACAGAUGAAGAAA	250	183	UUCUCAUCUGUAAAUG	290	
183	ACUGAAGCAUAGACCUGCU	251	183	ACUGAAGCAUAGACCUGCU	251	201	AGCAGGGCUAUGCUCUICAGU	291	
201	UUAUGUGAGAAACGCA	252	201	UUAUGUGAGAAACGCA	252	219	UGCGUUCUUCUACAUAA	292	
219	AGGGAGACAGUUCAGUCAC	253	219	AGGGAGACAGUUCAGUCAC	253	237	GUGACUGAACUGUCUCCCCU	293	
237	CUGCAAUUCUCAUGCCCCAU	254	237	CUGCAAUUCUCAUGCCCCAU	254	255	AUGGGCAUGAAGAUUGGCAG	294	
255	UCAGUUUUCUGUGAGAAGA	255	255	UCAGUUUUCUGUGAGAAGA	255	273	UCUUCUCAACAAAGAAACUGA	295	
273	AAAACAAGACUGGCAACGC	321	273	AAAACAAGACUGGCAACGC	321	291	GCGUJGGCAGCUUQJUJJU	359	
291	CCUGCUUCCUCUCUGAGC	322	291	CCUGCUUCCUCUCUGAGC	322	309	GCUCAGAGGGAGGAAGCAGG	360	
309	CUGUCAAGUAGGAAGGUCCG	323	309	CUGUCAAGUAGGAAGGUCCG	323	327	CGGACUUCUACUUGACAG	361	
327	GGGCUUCUCUGCUJAGAAA	324	327	GGGCUUCUCUGCUJAGAAA	324	345	CUUUCUAGCAGGCAGGCC	362	
345	GAGAAGCUAUGGCGAGG	325	345	GAGAAGCUAUGGCGAGG	325	363	CUCCUGACAUAGCUUCUC	363	
363	GCACUGAGGCAUCCCAGGU	326	363	GCACUGAGGCAUCCCAGGU	326	381	ACCUGGGGAUGGCCUCAGUGC	364	
381	UGUGACACUCUCCACC	327	381	UGUGACACUCUCCACC	327	399	UAGGUGGAAGAGUGUCAC	365	
399	AGAGCAUUCGUCUCUCAU	328	399	AGAGCAUUCGUCUCUCAU	328	417	AUGAGGAGACGGAAUGCUCU	366	
417	UCCUCUGCCAUUGGCCAUG	339	417	UCCUCUGCCAUUGGCCAUG	339	435	CAUGGCACAUAGGCAGAGGA	547	
435	GUUUUGAACCUUAGAUUA	340	435	GUUUUGAACCUUAGAUUA	340	453	UAAUCUJAGGGUUCAAAAC	548	
453	AGAGGGCUAAGCAUUCU	341	453	AGAGGGCUAAGCAUUCU	341	471	AGAAAUGGUUCUAGGCCUCU	549	
471	UGGGAAUJUACUCUGAAU	342	471	UGGGAAUJUACUCUGAAU	342	489	AUUCAGAGUAAAUAUCCAA	550	
489	UUCUACGUAGACCAUUC	343	489	UUCUACGUAGACCAUUC	343	507	GAAAUGGUUCUACGUAGAA	551	
507	CAUGGUUAUACCUUCUCU	344	507	CAUGGUUAUACCUUCUCU	344	525	CAGAGGGGUUAACACAU	552	
525	GAGUACCCUCAGGUAGGG	345	525	GAGUACCCUCAGGUAGGG	345	543	CCCUACCUAGGGGACUC	553	
530	ACCCUCAGGUAGGGACAUU	346	530	ACCCUCAGGUAGGGACAUU	346	548	AAUGGUCCUACCUAGGGGU	554	

## AAA1-8

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3	UAGGACUCAGAAAUAUAGA	241	3	UAGGACUCAGAAAUAUAGA	241	21	UCUAUAAAUCAGAGGUCCUA	281
21	AUGUUAGUAAGGCAAACA	242	21	AUGUUAGUAAGGCAAACA	242	39	UGUUUGCUUUACUAACAU	282
39	AGACAUAAACAGAUAAACACA	243	39	AGACAUAAACAGAUAAACACA	243	57	UGGUUAUCUGUAUGUCU	283
57	AUACAAAGUGCCUACCCACA	244	57	AUACAAAGUGCCUACCCACA	244	75	UGUGGUAGGGCACUUJGUUAU	284
75	AUGCUAAACCACUGGUCCAG	245	75	AUGCUAAACCACUGGUCCAG	245	93	CUGGAGCAGUGGUAGCAU	285
93	GGCACUUUCUAUAGAAGAA	246	93	GGCACUUUCUAUAGAAGAA	246	111	UUCUUCUAUAGAAAGUGGC	286
111	ACUAAUJUAUACUACCA	247	111	ACUAAUJUAUACUACCA	247	129	UGGUGAUGAUAAAUAUAGU	287
129	AUAACCCUAUGGGGUAGAU	248	129	AUAACCCUAUGGGGUAGAU	248	147	AUCUACCCCUAUGGGGUAU	288
147	UGAUUUUUJUACAACCUC	249	147	UGAUUUUUJUACAACCUC	249	165	GGAGGGUUGUAAAUAUCA	289
165	CAUUUACAGAUGAAGAAA	250	165	CAUUUACAGAUGAAGAAA	250	183	UUUCUUCAUUCGUAAAUAUG	290
183	ACUGAAAGCAUAGACCUGCU	251	183	ACUGAAAGCAUAGACCUGCU	251	201	AGCAGGUCUUAUGCUUCAGU	291
201	UUAUGUGAGAAGAAACGCA	252	201	UUAUGUGAGAAGAAACGCA	252	219	UGCGUUUCUUCACAUAA	292
219	AGGGAGACAGUUCAGUCAC	253	219	AGGGAGACAGUUCAGUCAC	253	237	GUGACUGAACUGUCUCCU	293
237	CUGCAAUCUUCAUAGCCAU	254	237	CUGCAAUCUUCAUAGCCAU	254	255	AUGGGCAUGAAGAUUGGAG	294
255	UCAGUUUCUUGUGAGAAGA	255	255	UCAGUUUCUUGUGAGAAGA	255	273	UCUUUCACAAAGAAACUGA	295
273	AAAACAAGUGGAUUAACAC	255	273	AAAACAAGUGGAUUAACAC	255	291	GUGUAUAUCCACUUGUUUU	560
291	CUGUCCAAAGGAGCAUGUG	256	291	CUGUCCAAAGGAGCAUGUG	256	309	CACAUJGCUGCUUGGAACAG	561
309	GUUGAAAAGAUUUUCUUU	257	309	GUUGAAAAGAUUUUCUUU	257	327	AAAGACAAAUCUUUCAAC	562
327	UUCCCCAUUAUGGUCUU	258	327	UUCCCCAUUAUGGUCUU	258	345	AAGACCAUAAAUGGGGAA	563
345	UGGUACCUUUCUCAAAAU	259	345	UGGUACCUUUCUCAAAAU	259	363	AUUIJUGGAAAGGUACCA	564
356	UCAAAAUUUGACCAUAU	221	356	UCAAAAUUUGACCAUAU	221	374	AUUAUJGGUCAUUUUUGA	240

## AAA1-9

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID	Seq ID
3	UGAUGGUGGAAGGAGAAUG	203	3	UGAUGGUGGAAGGAGAAUG	203	21	CAUUCUCCUUCACCAUCA	222	
21	GAGUCUCUGAUGCUCUUUGG	204	21	GAGUCUCUGAUGCUCUUUGG	204	39	CCAAAGGCCAUCAAGACUC	223	
39	GACUUGAUGCUGGAAAGAC	205	39	GACUUGAUGCUGGAAAGAC	205	57	GUCUUCCAGCAUCAAGUC	224	
57	CUUAAGACUUIUGGGGACU	206	57	CUUAAGACUUIUGGGGACU	206	75	AGUCCCCAAAGCUUAAAG	225	
75	UACUGGAAAGGAGUGACU	207	75	UACUGGAAAGGAGUGACU	207	93	AAGUCACUCCUUCUCCAGUA	226	
93	UCUCCCCAGAUUUUGUAU	208	93	UCUCCCCAGAUUUUGUAU	208	111	AUACAAAAAUUCUGGGGAGA	227	
111	UACCUUGACUCUGUUCAGC	209	111	UACCUUGACUCUGUUCAGC	209	129	GCUGAAACAGAGUCAGGUA	228	
129	CAUCCGCUUCCAAAAGAAU	210	129	CAUCCGCUUCCAAAAGAAU	210	147	AUUCUUIJGGGAAGGGGAUG	229	
147	UGCAUGUGAAGCAGGAGC	211	147	UGCAUGUGAAGCAGGAGC	211	165	GCUCUCUGCUUCAACUGCA	230	
165	CUUAUGUGAAGAAACGC	212	165	CUUAUGUGAAGAAACGC	212	183	GGGUUUCUUCUCAUAUAAAG	231	
183	CAGGGAGACAGUUCAGUCA	213	183	CAGGGAGACAGUUCAGUCA	213	201	UGACUGAACUGUCUCCUG	232	
201	ACUGCAAAUCUUCAUGCCCCA	214	201	ACUGCAAAUCUUCAUGCCCCA	214	219	UGGGCAUGAAAGAUUGCAGU	233	
219	AUCAGUUUCUGUGAGAA	215	219	AUCAGUUUCUGUGAGAA	215	237	CUUCUCAAGAAACUGAU	234	
237	GAAAACAAGUUUAGAAAAA	237	GAAAACAAGUUUAGAAAAA	237	565	UUUUCCUAAACUJGUUUUUC	573		
255	ACUUCCUACACCUUCUUUG	566	255	ACUUCCUACACCUUCUUUG	566	273	CAAAGAAGGGUJAGGAAGU	574	
273	GUUGGGGAUGUUUCUGGAC	567	273	GUUGGGGAUGUUUCUGGAC	567	291	GUCCAGAGAACAUCCCAAC	575	
291	CUUAUGACUCCAGGGAGA	568	291	CUUAUGACUCCAGGGAGA	568	309	UCUCGCCUGGAGUCAUUAG	576	
309	ACCAACGUUGUAUAGAAC	569	309	ACCAACGUUGUAUAGAAC	569	327	GUUCAUGAUCAACGGGGGU	577	
327	CUCACUUUGAAACAGAAAGC	570	327	CUCACUUUGAAACAGAAAGC	570	345	GCUUUCUGUUUCAAAAGUGAG	578	
345	CUGGGUUGGUAGACUGGA	571	345	CUGGGUUGGUAGACUGGA	571	363	UCCAGUCUUACCAACCCAG	579	
349	GUUGGUAAAGACUGGGACUA	572	349	GUUGGUAAAGACUGGGACUA	572	367	UAGCUCCAGUCUUACCAAC	580	

The 3'-ends of the Upper sequence and the Lower sequence of the siRNA construct can include an overhang sequence, for example about 1, 2, 3, or 4 nucleotides in length, preferably 2 nucleotides in length, wherein the overhanging sequence of the lower sequence is optionally complementary to a portion of the target sequence. The upper sequence is also referred to as the sense strand, whereas the lower sequence is also referred to as the antisense strand. The upper and lower sequences in the Table can further comprise a chemical modification having Formulae I-VII, such as exemplary siRNA constructs shown in Figures 4 and 5, or having modifications described in Table IV or any combination thereof.

TABLE III: GPR154 and AAA1-4 Synthetic Modified siNA Constructs

GPR154

Target Pos	Target	Cmpd #	Aliases	Sequence	Seq ID
200	GGCUUJGCACUGAAAACAGUGACUU	581	GPR154:202U21 sense siNA	CUUCCACUGAAAACAGUGACTT	597
399	GCCAUACAGAUUUCUUCACAGG	582	GPR154:401U21 sense siNA	CAUCACAGAUUUCUUCACATT	598
867	GUGAUUJCCAAUCUGCUAGAUGG	583	GPR154:869U21 sense siNA	GAUUCUCCAAUCUGCUAGAUGT	599
888	GGGAAACUGUGCAGCAGCUAA	584	GPR154:890U21 sense siNA	GAACUGUGCAGCAGCUAATT	600
899	CAGCAGCUAAACCGAGGACUCA	585	GPR154:901U21 sense siNA	GCAGCUUAAACCGAGGACUTT	601
921	AUCUCAAAGGCAAAAUCAAGGC	586	GPR154:923U21 sense siNA	CUCAAAGGCAAAAUCAAGTT	602
965	CAUUCUJGCCUUCUCAUCUGCUUU	587	GPR154:967U21 sense siNA	UUCUJGCCUUCUCAUCUGCUGTT	603
1021	AUUCUCAACCUUCUCCAGAACACC	588	GPR154:1023U21 sense siNA	UUCAACCUUCUCCAGAACATT	604
200	GGCUUJGCACUGAAAACAGUGACUU	581	GPR154:220L21 antisense siNA (202C)	GUACUGUUUUCAGUGCAAGTT	605
399	GCCAUACAGAUUUCUUCACAGG	582	GPR154:419L21 antisense siNA (401C)	UGUGAAAAGAAUCUGUGAUGTT	606
867	GUGAUUJCCAAUCUGCUAGAUGG	583	GPR154:887L21 antisense siNA (869C)	AUCUGAGCAGUUGGAAAUCTT	607
888	GGGAAACUGUGCAGCAGCUAA	584	GPR154:908L21 antisense siNA (890C)	AUAGCUGUGCAGCAGUUJUUCTT	608
899	CAGCAGCUAAACCGAGGACUCA	585	GPR154:919L21 antisense siNA (901C)	AGUCCUCGGGUUAUAGCUGCTT	609
921	AUCUCAAAGGCAAAAUCAAGGC	586	GPR154:941L21 antisense siNA (923C)	CUUCAUUUJGCCUUIUAGTT	610
965	CAUUCUJGCCUUCUCAUCUGCUUU	587	GPR154:985L21 antisense siNA (967C)	CAGCAGAUAGGCAAGAATT	611
1021	AUUCUCAACCUUCUCCAGAACACC	588	GPR154:1041L21 antisense siNA (1023C)	UGUCUGGAAGGGAGGUUGUAATT	612
200	GGCUUJGCACUGAAAACAGUGACUU	581	GPR154:202U21 sense siNA stab04	B cuuGcAcuGAAAACAGUGACTT B	613
399	GCCAUACAGAUUUCUUCACAGG	582	GPR154:401U21 sense siNA stab04	B cuucAcAGAucuucuucAcATT B	614
867	GUGAUUJCCAAUCUGCUAGAUGG	583	GPR154:869U21 sense siNA stab04	B GAuucuccAAcucGcucAGAUtt B	615
888	GGGAAACUGUGCAGCAGCUAA	584	GPR154:890U21 sense siNA stab04	B GAAAcuGuGcAGGcAGCUAUtt B	616
899	CAGCAGCUAAACCGAGGACUCA	585	GPR154:901U21 sense siNA stab04	B GcAGCuuAAACccGAGGAcuTT B	617
921	AUCUCAAAGGCAAAAUCAAGGC	586	GPR154:923U21 sense siNA stab04	B cuuAAAGGCAAAAUcaAGTT B	618
965	CAUUCUJGCCUUCUCAUCUGCUUU	587	GPR154:967U21 sense siNA stab04	B uucuucuucuucuucuucuucuuc B	619
1021	AUUCUCAACCUUCUCCAGAACACC	588	GPR154:1023U21 antisense siNA (202C)	B uucAAAcccuucuucAGAcATT B	620
200	GGCUUJGCACUGAAAACAGUGACUU	581	stab05	GPR154:419L21 antisense siNA (401C)	stab05
399	GCCAUACAGAUUUCUUCACAGG	582		UGUAAAAGAAUuGcAGGAGUuGTT	621

867	GUGAUUUCCAACUGCUCAAGAUGG	583	GPR154:887L21 antisense siNA (869C) stab05	AucuGAGcAGGuuGGAAAAGAATst	623
888	GGGAAACUGUGGAGGAGCUAAUA	584	GPR154:908L21 antisense siNA (890C) stab05	AuAGcuGcuGcAcAGuuuuctst	624
899	CAGCAGCUAUACCAGGACUCA	585	GPR154:919L21 antisense siNA (901C) stab05	AGuccuGGuuAuAGcuGctst	625
921	AUCUCAAAGGCAAAAUCAAGGC	586	GPR154:941L21 antisense siNA (923C) stab05	cuuGAuuuuGcuuuGAGtst	626
965	CAUUCUJGCCUJUCAUCUGCUGUU	587	GPR154:985L21 antisense siNA (967C) stab05	cAGcAGAUGAAGGcAAAGAATst	627
1021	AUUUCAACCUCCUCCAGACACC	588	GPR154:1041L21 antisense siNA (1023C) stab05	uGuclGGAAAGGGGuuGAATst	628
200	GGCUCUGCACUUGAAAACAGUGACUU	581	GPR154:202U21 sense siNA stab07	B cuuGcAcuGAAAACAGGuGactt B	629
399	GCCAUJACAGAUJCUUUCACAGG	582	GPR154:401U21 sense siNA stab07	B AucAcAGAuucuuucACATT B	630
867	GUGAUUUCCAACUGCUCAAGAUGG	583	GPR154:3869U21 sense siNA stab07	B GAuucuCCACuGcuAGAUtt B	631
888	GGGAAACUGUGGAGGAGCUAAUA	584	GPR154:890U21 sense siNA stab07	B GAAAcuGuGcAGGcGuAUtt B	632
899	CAGCAGCUAUACCAGGACUCA	585	GPR154:901U21 sense siNA stab07	B GcAGcuAuAAAccGGGGAcUtt B	633
921	AUCUCAAAGGCAAAAUCAAGGC	586	GPR154:923U21 sense siNA stab07	B cuuAAAGGcAAAAAAGcuAGtt B	634
965	CAUUCUJGCCUJUCAUCUGCUGUU	587	GPR154:967U21 sense siNA stab07	B uuucuuGcuuucAucuGcuGtt B	635
1021	AUUUCAACCUCCUCCAGACACC	588	GPR154:1023U21 sense siNA stab07	B uuucAAccuCCuuccAGAcATT B	636
200	GGCUJGGCACUGAAACAGUGACUU	581	GPR154:220L21 antisense siNA (202C) stab11	GucauGuuucAGuGcAAgtst	637
399	GCCAUJACAGAUJCUUUCACAGG	582	GPR154:419L21 antisense siNA (401C) stab11	uGuGAAAGAAuucuGuGAUGtst	638
867	GUGAUUUCCAACUGCUCAAGAUGG	583	GPR154:3887L21 antisense siNA (869C) stab11	AucuGAGcAGGuuGGAAAAGtst	639
888	GGGAAACUGUGGAGGAGCUAAUA	584	GPR154:908L21 antisense siNA (890C) stab11	AuAGcuGcuGcAcAGuuuctst	640
899	CAGCAGCUAUACCAGGACUCA	585	GPR154:919L21 antisense siNA (901C) stab11	AGuccuGGuuAuAGcuGctst	641
921	AUCUCAAAGGCAAAAUCAAGGC	586	GPR154:941L21 antisense siNA (923C) stab11	cuuGAuuuuGcuuuGAGtst	642
965	CAUUCUJGCCUJUCAUCUGCUGUU	587	GPR154:985L21 antisense siNA (967C) stab11	cAGcAGAUGAAGGcAAAGAATst	643
1021	AUUUCAACCUCCUCCAGACACC	588	GPR154:1041L21 antisense siNA (1023C) stab11	uGuuGGAAAGGGGuuGAATst	644
200	GGCUJGGCACUGAAACAGUGACUU	581	GPR154:202U21 sense siNA stab18	B cuuGcAcuGAAAAGGuGactt B	645
399	GCCAUJACAGAUJCUUUCACAGG	582	GPR154:401U21 sense siNA stab18	B AucAcAGAuucuuucACATT B	646
867	GUGUUUUCCAACUGCAGAAGG	583	GPR154:869U21 sense siNA stab18	B GAuuuuuACAGGuuACATT B	647

888	GGGAAACUGUGGAGGCAGCUAUAA	584	GPR154:890U21 sense siNA stab18	B GAAA <u>AcuGuGcAGcAGcAU</u> TT B	648
889	CAGCAGCUUAACCGAGGACUCA	585	GPR154:901U21 sense siNA stab18	B GcAG <u>CUuAAAccGAGGAC</u> TT B	649
921	AUCUCAAGGCAAAUCAAGGC	586	GPR154:923U21 sense siNA stab18	B <u>cuuAAAAGGcAAAAA</u> u <u>uAAG</u> TT B	650
965	CAUUCUJGCCUUCAUCUGCUGUU	587	GPR154:967U21 sense siNA stab18	B <u>uicu</u> u <u>Gccu</u> u <u>AcuG</u> u <u>G</u> TT B	651
1021	AUUUCAACCUCUUCAGACACC	588	GPR154:1023U21 sense siNA stab18	B <u>uicA</u> Acc <u>uccu</u> u <u>ccAG</u> AcATT B	652
200	GGCUUJGACUGAAACAGUGACUU	581	GPR154:220L21 antisense siNA (202C)		
399	GCCAUCAACAGAUUUUCACAGG	582	GPR154:887L21 antisense siNA (869C)	G <u>AcuGu</u> u <u>uAcG</u> u <u>GcAA</u> GtST	653
867	GUGAUUUCCAACUGCUAGAUGG	583	GPR154:419L21 antisense siNA (401C)	<u>uGuGAA</u> AGAA <u>AcuGuG</u> AuGTst	654
888	GGGAAACUGUGGAGGCAGCUAUAA	584	GPR154:908L21 antisense siNA (890C)	Au <u>AGc</u> u <u>GuGcAcA</u> Guu <u>u</u> u <u>c</u> T	655
899	CAGCAGCUUAACCGAGGACUCA	585	GPR154:919L21 antisense siNA (901C)	Au <u>AGc</u> u <u>GuGcAcA</u> Guu <u>u</u> u <u>c</u> T	656
921	AUCUCAAGGCAAAUCAAGGC	586	GPR154:941L21 antisense siNA (923C)	AG <u>uccu</u> GG <u>GuuAuA</u> G <u>GuG</u> CtST	657
965	CAUUCUJGCCUUCAUCUGCUGUU	587	GPR154:985L21 antisense siNA (967C)	cuu <u>Gauuuu</u> G <u>ccuuu</u> GAGTst	658
1021	AUUUCAACCUCUUCAGACACC	588	GPR154:1041L21 antisense siNA (1023C)	cAG <u>AG</u> u <u>GAAGG</u> AA <u>GA</u> ATst	659
200	GGCUUJGACUGAAACAGUGACUU	581	GPR154:202U21 sense siNA stab09	<u>uGu</u> u <u>GuGAGGAGG</u> u <u>GAAT</u> st	660
399	GCCAUCAACAGAUUUUCACAGG	582	GPR154:401U21 sense siNA stab09	B CU <u>UG</u> AC <u>UGAA</u> ACAG <u>UGAC</u> TT B	661
867	GUGAUUUCCAACUGCUAGAUGG	583	GPR154:869U21 sense siNA stab09	B CAU <u>CA</u> G <u>AUUUC</u> U <u>UAC</u> ATT B	662
888	GGGAAACUGUGGAGGAGCUAUAA	584	GPR154:890U21 sense siNA stab09	B GAU <u>UCC</u> AA <u>ACUG</u> CU <u>GA</u> U <u>TT</u> B	663
899	CAGCAGCUUAACCGAGGACUCA	585	GPR154:901U21 sense siNA stab09	B GAA <u>ACU</u> G <u>UG</u> AG <u>CG</u> AG <u>CU</u> U <u>TT</u> B	664
921	AUCUCAAGGCAAAUCAAGGC	586	GPR154:923U21 sense siNA stab09	B GCAG <u>CU</u> UA <u>ACCG</u> AG <u>GA</u> U <u>TT</u> B	665
965	CAUUCUJGCCUUCAUCUGCUGUU	587	GPR154:967U21 sense siNA stab09	B CUC <u>AA</u> AG <u>GG</u> AA <u>AA</u> u <u>u</u> u <u>CAAG</u> TT B	666
1021	AUUUCAACCUCUUCAGACACC	588	GPR154:1023U21 sense siNA stab09	B UU <u>CU</u> U <u>GU</u> U <u>U</u> u <u>UCC</u> AG <u>AC</u> TT B	667
200	GGCUUJGACUGAAACAGUGACUU	581	GPR154:220L21 antisense siNA (202C)	GU <u>ACU</u> GU <u>U</u> U <u>U</u> u <u>U</u> AG <u>GA</u> GTst	668
399	GCCAUCAACAGAUUUUCACAGG	582	GPR154:419L21 antisense siNA (401C)	UG <u>UGAA</u> AGAA <u>U</u> U <u>U</u> u <u>U</u> u <u>U</u> UG <u>AU</u> GTst	669
867	GUGAUUUCCAACUGCUAGAUGG	583	GPR154:887L21 antisense siNA (869C)	AUC <u>U</u> GG <u>AC</u> GU <u>U</u> GG <u>AA</u> U <u>U</u> CTst	670
888	GGGAAACUGUGGAGGAGCUAUAA	584	GPR154:908L21 antisense siNA (890C)	AU <u>AGC</u> U <u>U</u> GG <u>U</u> GG <u>AC</u> GU <u>U</u> U <u>U</u> CTst	671
899	CAGCAGCUUAACCGAGGACUCA	585	GPR154:919L21 antisense siNA (901C)	AG <u>UCC</u> U <u>GG</u> UU <u>U</u> AG <u>CU</u> U <u>U</u> GG <u>CT</u> st	672

921	AUCUCAAAGGCAAAAUCAGGC	586	GPR154:941L21 antisense siNA (923C) stab10	CUUGAUUUUUGCCUUUUGAGTST	674
965	CAUUCUUGCCUUCAUCUGCUGUU	587	GPR154:985L21 antisense siNA (967C) stab10	CAGCAGAUGAAGGCAAGAATST	675
1021	AUUCUACCUCCUCCAGACACC	588	GPR154:1041L21 antisense siNA (1023C) stab10	UGUCUGGAAGGGAGGUGAATST	676
200	GGCUUUGCACUGAAACAGUGACUU	581	GPR154:220L21 antisense siNA (202C) stab19	GuAcuGuuucAGuGcAAGTT B	677
399	GCCCAUCACAGAUUCUUCACAGG	582	GPR154:419L21 antisense siNA (401C) stab19	uGuGAAAGAAucuGuGauGTT B	678
867	GUGAUUUCCAACUGCUAGAUGG	583	GPR154:987L21 antisense siNA (869C) stab19	AucuGAGGAGuGGAAuCTT B	679
888	GGGAAACUGUGGCAGCUAUAA	584	GPR154:908L21 antisense siNA (890C) stab19	AuAGcuiGcAcAGcuiucTT B	680
899	CAGCAGCUAUAAACCGGACUCA	585	GPR154:919L21 antisense siNA (901C) stab19	AGucccGGuAUAGcugGcTT B	681
921	AUCUCAAAGGCAAAAUCAGGC	586	GPR154:941L21 antisense siNA (923C) stab19	cuuGAuuuuGccuuuGAGTT B	682
965	CAUUCUUGCCUUCAUCUGCUGUU	587	GPR154:985L21 antisense siNA (967C) stab19	cAGcAGAUGAAGGCAAGAATT B	683
1021	AUUCUACCUCCUCCAGACACC	588	GPR154:1041L21 antisense siNA (1023C) stab19	uGucuGGAAAGGGGUUGAATT B	684
200	GGCUUUGCACUGAAACAGUGACUU	581	GPR154:220L21 antisense siNA (202C) stab22	GUACACUGUUCAGUGCAAGTT B	685
399	GCCAUACAGAUUCUUCACAGG	582	GPR154:419L21 antisense siNA (401C) stab22	UGUGAAAGAAUCUGUGAUGTT B	686
867	GUGAUUUCCAACUGCUAGAUGG	583	GPR154:987L21 antisense siNA (869C) stab22	AUCUGAGGAGUUGAAUUCTT B	687
888	GGGAAACUGUGGCAGCUAUAA	584	GPR154:908L21 antisense siNA (890C) stab22	AUAGCUGUGUGCACAGUUUCTT B	688
899	CAGCAGCUAUACCGGAGACUCA	585	GPR154:919L21 antisense siNA (901C) stab22	AGUCCUCGGUUAUGCUGCTT B	689
921	AUCUCAAAGGCAAAAUCAGGC	586	GPR154:941L21 antisense siNA (923C) stab22	CUUGAUUUUGCCUUUUGAGTT B	690
965	CAUUCUUGCCUUCAUCUGCUGUU	587	GPR154:985L21 antisense siNA (967C) stab22	CAGCAGAUGAAGGCAAGAATT B	691
1021	AUUCUACCUCCUCCAGACACC	588	GPR154:1041L21 antisense siNA (1023C) stab22	UGUCUGGAAGGGAGGUUAATT B	692

## AAA1-4

Target Pos	Target	Seq ID	Cmpd #	Aliases	Sequence	Sq ID
62	AAGUGCCUACCAUGCUAACCA	589		AAA1-4:64U21 sense siNA	GUGCCUACCAUGCUAACCTT	693
394	CACCUAGGCAUCCGUCUCUCA	590		AAA1-4:396U21 sense siNA	CCUAGGCAUCCGUCUCUTT	694
410	UCUCUCAUCCUCUGCCAUGUAGC	591		AAA1-4:412U21 sense siNA	UCUCAUCCUCUGCCAUGUATT	695
434	AACUGCUAUGCUCUUCAGCUG	592		AAA1-4:436U21 sense siNA	CUGCUAUGCUCUUCAGCUTT	696
640	GACUUUUCAAGCCCAGAUGU	593		AAA1-4:642U21 sense siNA	CUUUUCAAGCCCAGAUGUTT	697
671	CCAUUCUCAAGGAUCUGAUUU	594		AAA1-4:673U21 sense siNA	AUUCUCAAGGAUCUGGAUUTT	698
873	AAUGACACCCUUGGAUCUGAAC	595		AAA1-4:875U21 sense siNA	UGACACCCUUGGAUCUGAAATT	699
897	UACACACCAGACAAUUGUGUGC	596		AAA1-4:899U21 sense siNA	CACACCAAGGACAAUUGUGUTT	700
62	AAGUGCCUACCAUGCUAACCA	589		AAA1-4:82U21 antisense siNA (64C)	GUUAGCAUGUGGUAGGCACTT	701
394	CACCUAGGCAUCCGUCUCUCA	590		AAA1-4:414L21 antisense siNA (396C)	AGAGACGGAAUGCUCUAGGTT	702
410	UCUCUCAUCCUCUGCCAUGUAGC	591		AAA1-4:430L21 antisense siNA (412C)	UACAUGGCAGAGGAUGAGATT	703
434	AACUGCUAUGCUCUUCAGCUG	592		AAA1-4:454L21 antisense siNA (436C)	GCUGAAGGGAUGCAUAGCAGTT	704
640	GACUUUUCAAGCCCAGAUGU	593		AAA1-4:660L21 antisense siNA (642C)	AUCUUGGGCUUGAAAAAGTT	705
671	CCAUUCUCAAGGAUCUGAUUU	594		AAA1-4:691L21 antisense siNA (673C)	AUCGAGAUCCUUGGAGAAUUTT	706
873	AAUGACACCCUUGGAUCUGAAC	595		AAA1-4:893L21 antisense siNA (875C)	UUCAGAUCCAAGGGUGUCATT	707
897	UACACACCAGACAAUUGUGUGC	596		AAA1-4:917L21 antisense siNA (899C)	ACACAAUUGUCCUUGGUGUTT	708
62	AAGUGCCUACCAUGCUAACCA	589		AAA1-4:64U21 sense siNA stab04	B GuGccuAccAcuGcuAACTT B	709
394	CACCUAGGCAUCCGUCUCUCA	590		AAA1-4:396U21 sense siNA stab04	B ccuAGGAcuUccGcuUcUTT B	710
410	UCUCUCAUCCUCUGCCAUGUAGC	591		AAA1-4:412U21 sense siNA stab04	B ucuGcAccuGcuGccAuGuATT B	711
434	AACUGCUAUGCUCUUCAGCUG	592		AAA1-4:436U21 sense siNA stab04	B cuGcuAuGcAccuGcUAGcTT B	712
640	GACUUUUCAAGCCCAGAUGU	593		AAA1-4:642U21 sense siNA stab04	B cuuuuuuGcccaAGGAUUTT B	713
671	CCAUUCUCAAGGAUCUGAUUU	594		AAA1-4:673U21 sense siNA stab04	B AuucuGcAccGGAuGcUcGAUUTT B	714
873	AAUGACACCCUUGGAUCUGAAC	595		AAA1-4:875U21 sense siNA stab04	B GcAcAccUuGGGAcuGAAUTT B	715
897	UACACACCAGACAAUUGUGUGC	596		AAA1-4:899U21 sense siNA stab04	B cACACAGGACAAuGuGUTT B	716
62	AAGUGCCUACCAUGCUAACCA	589		AAA1-4:82L21 antisense siNA (64C) stab05	GuuAGcAuGuGGGuAGGcActT	717
394	CACCUAGGCAUCCGUCUCUCA	590		AAA1-4:414L21 antisense siNA (396C) stab05	AGAGACGGAAuGcuuAGGTst	718
410	UCUCUCAUCCUCUGCCAUGUAGC	591		AAA1-4:430L21 antisense siNA (412C) stab05	uAcAuGGcAGGGGuAGGATst	719
434	AACUGCUAUGCUCUUCAGCUG	592		AAA1-4:454L21 antisense siNA (436C) stab05	GuuGAAAGGAAuGcAGGTst	720
640	GACUUUUCAAGCCCAGAUGU	593		AAA1-4:660L21 antisense siNA (642C) stab05	AucuuGGGcuuGAAAAGTst	721

671	CCAUUCUCAAGGAUCUCGAUUU	594	AAA14:691L21 antisense siNA (673C) stab05	AucGAGAUCUUGGAGAAUdT	722
873	AAUGACACCCUUGGAUCUGAACAA	595	AAA14:893L21 antisense siNA (875C) stab05	uucAGAUccAAAGGGuGucATdT	723
897	UACACCCGGACAAUUGUGUGC	596	AAA14:917L21 antisense siNA (899C) stab05	AcACAAuGuccGGuGuGtT	724
62	AAGUGCCUACCAAGCUAACCA	599	AAA14:64U21 sense siNA stab07	B GuGccAAccAcAuGcuaAcTT B	725
394	CACCUAGAGCAUCCGUCUCUCA	590	AAA14:396U21 sense siNA stab07	B ccuAGAGcAuuccGcucuTT B	726
410	UCUCUCAUCCUUCUGCCAUQAGC	591	AAA14:412U21 sense siNA stab07	B ucucAuuccuGccAuGuATT B	727
434	AACUUGCUAUGGCAUCCUUCAGCUG	592	AAA14:436U21 sense siNA stab07	B cuGcuAUGcAuuccuAGcTT B	728
640	GACUUUUUCAAGCCCCAAAGAUGU	593	AAA14:642U21 sense siNA stab07	B cuuuucaAGccCAAGAUdT B	729
671	CCAUUCUCAAGGAUCUCGAUUU	594	AAA14:673U21 sense siNA stab07	B AuucucuAGGAuucGcAUdT B	730
873	AAUGACACCCUUGGAUCUGAACAA	595	AAA14:875U21 sense siNA stab07	B uGAcAcuGuGGAUcGuGAATT B	731
897	UACACCCGGACAAUUGUGUGC	596	AAA14:899U21 sense siNA stab07	B cACACAGGACAAuGuGuTT B	732
62	AAGUGCCUACCAAGCUAACCA	599	AAA14:82L21 antisense siNA (64C) stab11	GuuAGcAUGuGGGUAGGcAcTdT	733
394	CACCUAGAGCAUCCGUCUCUCA	590	AAA14:414L21 antisense siNA (396C) stab11	AGAGAGGGAAuGcucuAGGdT	734
410	UCUCUCAUCCUUCGGCAUGUAGC	591	AAA14:430L21 antisense siNA (412C) stab11	uACAUGGcAGAGGGAUAGAGdT	735
434	AACUUGCUAUGGCAUCCUUCAGCUG	592	AAA14:454L21 antisense siNA (436C) stab11	GuuGAGGAuGcAUAGcAGdT	736
640	GACUUUUUCAAGCCCCAAAGAUGU	593	AAA14:660L21 antisense siNA (642C) stab11	AucuuGGGGcuuGAAAAGdT	737
671	CCAUUCUCAAGGAUCUCGAUUU	594	AAA14:691L21 antisense siNA (673C) stab11	AucGAGAUCUuGGAGAAudT	738
873	AAUGACACCCUUGGAUCUGAACAA	595	AAA14:893L21 antisense siNA (875C) stab11	uucAGAUccAAAGGGuGucATdT	739
897	UACACCCGGACAAUUGUGUGC	596	AAA14:917L21 antisense siNA (899C) stab11	AcACAAuGuccGGuGuGtT	740
62	AAGUGCCUACCAAGCUAACCA	597	AAA14:64U21 sense siNA stab18	B GuGccAAccAcAuGcuaAcTT B	741
394	CACCUAGAGCAUCCGUCUCUCA	590	AAA14:396U21 sense siNA stab18	B ccuAGAGcAuuccGcucuTT B	742
410	UCUCUCAUCCUUCGGCAUGUAGC	591	AAA14:412U21 sense siNA stab18	B ucucAuuccuGccAuGuATT B	743
434	AACUUGCUAUGGCAUCCUUCAGCUG	592	AAA14:436U21 sense siNA stab18	B cuGcuAUGcAuuccuAGcTT B	744
640	GACUUUUUCAAGCCCCAAAGAUGU	593	AAA14:642U21 sense siNA stab18	B cuuuucaAGccCAAGAUdT B	745
671	CCAUUCUCAAGGAUCUCGAUUU	594	AAA14:673U21 sense siNA stab18	B AuucucuAGGAuucGcAUdT B	746
873	AAUGACACCCUUGGAUCUGAACAA	595	AAA14:875U21 sense siNA stab18	B uGAcAcuGuGGAUcGuGAATT B	747
897	UACACCCGGACAAUUGUGUGC	596	AAA14:899U21 sense siNA stab18	B cACACAGGACAAuGuGuTT B	748
62	AAGUGCCUACCAAGCUAACCA	599	AAA14:82L21 antisense siNA (64C) stab08	GuuAGcAUGuGGGUAGGcAcTdT	749

394	CACCUAGAGCAUCCGUCUCUCA	590	AAA1-4:141L21 antisense siNA (396C) stab08	AGAGAcGGAAU <u>G</u> cu <u>U</u> AGGTsT	750
410	UCUCUCAUCCUCAUGUAGC	591	AAA1-4:430L21 antisense siNA (412C) stab08	uAcAuGG <u>G</u> AGGG <u>A</u> AGGATsT	751
434	AAUCUGCUAUGC <u>U</u> UCAAGCUG	592	AAA1-4:454L21 antisense siNA (436C) stab08	Gu <u>U</u> AGGA <u>G</u> u <u>G</u> Au <u>G</u> AGTsT	752
640	GACUUUUUCAAGCCCCAACGAUGU	593	AAA1-4:660L21 antisense siNA (642C) stab08	A <u>U</u> uGG <u>G</u> Gu <u>U</u> GA <u>AA</u> AGTsT	753
671	CCAUUUCCAAAGGAUCUCGAAUU	594	AAA1-4:691L21 antisense siNA (673C) stab08	A <u>U</u> CG <u>G</u> Au <u>U</u> uGG <u>G</u> AAuTsT	754
873	AAUGACACCCUJGG <u>A</u> UCLGAACA	595	AAA1-4:893L21 antisense siNA (875C) stab08	u <u>U</u> CA <u>U</u> u <u>U</u> GG <u>G</u> u <u>U</u> CATsT	755
897	UACACACC <u>G</u> ACAA <u>U</u> GGUGGC	596	AAA1-4:917L21 antisense siNA (899C) stab08	Ac <u>U</u> Ac <u>U</u> u <u>U</u> Gu <u>U</u> GG <u>G</u> Gu <u>U</u> CATsT	756
62	AAGUGCCUAC <u>U</u> AC <u>U</u> GG <u>U</u> ACCA	589	AAA1-4:641U21 sense siNA stab09	B GU <u>U</u> GU <u>U</u> CC <u>U</u> AC <u>U</u> GG <u>U</u> u <u>U</u> ACTTsT	757
394	CACCUAGAGCAUCCGUCUCUCA	590	AAA1-4:396L21 sense siNA stab09	B CC <u>U</u> AG <u>U</u> GC <u>U</u> AU <u>U</u> CC <u>U</u> GU <u>U</u> TT	758
410	UCUCUCAUCCUCAUG <u>U</u> GUAGC	591	AAA1-4:412L21 sense siNA stab09	B UC <u>U</u> CA <u>U</u> CC <u>U</u> CG <u>U</u> CC <u>U</u> GU <u>U</u> TT	759
434	AAUCUGCUAUGC <u>U</u> UCAAGCUG	592	AAA1-4:436L21 sense siNA stab09	B CG <u>U</u> CG <u>U</u> AG <u>U</u> GC <u>U</u> CC <u>U</u> U <u>U</u> AGCTTsT	760
640	GACUUUUCAAGCCCCAACGAUGU	593	AAA1-4:642L21 sense siNA stab09	B CU <u>U</u> U <u>U</u> CA <u>U</u> AG <u>U</u> CC <u>U</u> AGAU <u>U</u> TT	761
671	CCAUUUCCAA <u>U</u> GAUCUG <u>U</u> UU	594	AAA1-4:673L21 sense siNA stab09	B AU <u>U</u> U <u>U</u> CC <u>U</u> AG <u>U</u> AC <u>U</u> CGAU <u>U</u> TT	762
873	AAUGACACCCUJGG <u>A</u> UCLGAACA	595	AAA1-4:875L21 sense siNA stab09	B UGACACCCUJGG <u>A</u> U <u>U</u> CGAU <u>U</u> ATT	763
897	UACACACC <u>G</u> ACAA <u>U</u> GGUGGC	596	AAA1-4:899L21 sense siNA stab09	B CAC <u>U</u> CC <u>U</u> GG <u>U</u> AC <u>U</u> U <u>U</u> GU <u>U</u> TT	764
62	AAGUGCCUAC <u>U</u> AC <u>U</u> GG <u>U</u> ACCA	589	AAA1-4:82L21 antisense siNA (64C) stab10	GU <u>U</u> AG <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> ACTsT	765
394	CACCUAGAGCAUCCGUCUCUCA	590	AAA1-4:141L21 antisense siNA (396C) stab10	AGAG <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> GGTsT	766
410	UCUCUCAUCCUCAUG <u>U</u> AGC	591	AAA1-4:430L21 antisense siNA (412C) stab10	U <u>U</u> CA <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> AGATsT	767
434	AAUCUGCUAUGC <u>U</u> UCAAGCUG	592	AAA1-4:454L21 antisense siNA (436C) stab10	GU <u>U</u> GA <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> AGTsT	768
640	GACUUUUCAAGCCCCAACGAUGU	593	AAA1-4:660L21 antisense siNA (642C) stab10	A <u>U</u> CU <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> AAAGTsT	769
671	CCAUUUCCAA <u>U</u> GAUCUG <u>U</u> UU	594	AAA1-4:691L21 antisense siNA (673C) stab10	A <u>U</u> CG <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> AAUTsT	770
873	AAUGACACCCUJGG <u>A</u> UCLGAACA	595	AAA1-4:893L21 antisense siNA (899C) stab10	ACAC <u>U</u> U <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> GGTsT	772
897	UACACACC <u>G</u> ACAA <u>U</u> GGUGGC	596	AAA1-4:82L21 antisense siNA (64C) stab19	Gu <u>U</u> AG <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> ACTsT	773
62	AAGUGCCUAC <u>U</u> AC <u>U</u> GG <u>U</u> ACCA	589	AAA1-4:414L21 antisense siNA (396C) stab19	AGAG <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> GG <u>U</u> GGTT	774
394	CACCUAGAGCAUCCGUCUCUCA	590			

		stab19	AAA1-4:430L21 antisense siNA (412C)	
410	UCUCUCAUCCUCUGCCAUGUAGC	591	AAA1-4:454L21 antisense siNA (436C)	uAcAUGGcAGAGGAuGAGATT B
434	AACUGCUAUGCACUCCUUCAGCUG	592	AAA1-4:660L21 antisense siNA (642C)	GcuGAAGGAuGcAuAGcAGTT B
640	GACUUUUUCAAGCCCCAAAGAUGU	593	stab19	AuciuiGGGcuiGAAAAAGTT B
671	CCAUUCUCCAAAGGAUCUCGAAUU	594	AAA1-4:691L21 antisense siNA (673C)	AucGAGAucuuiGGAGAAuTT B
873	AAUGACACCCUUGGAUCUGAACCA	595	AAA1-4:893L21 antisense siNA (875C)	uucAGAcuCCAGGGGcGicGATT B
897	UACACACCGGACAAUUGUGUGC	596	stab19	AcACAAuGuccuGuGuGTT B
62	AAGUGCCUACACAAUGUACAAACCA	589	AAA1-4:82L21 antisense siNA (64C)	GUUAAGCAUGGGGUAGGCACTT B
394	CACCUAGAGGAUUCGUUCUCA	590	stab22	AAA1-4:414L21 antisense siNA (396C)
410	UCUCUCAUCCUCUGCCAUGUAGC	591	AAA1-4:430L21 antisense siNA (412C)	AGAGACGGAAUGCUCUAGGTT B
434	AACUGCUAUGCACUCCUUCAGCUG	592	stab22	AAA1-4:454L21 antisense siNA (436C)
640	GACUUUUUCAAGCCCCAAAGAUGU	593	AAA1-4:660L21 antisense siNA (642C)	UACAUGGcAGGGGAUGAGATT B
671	CCAUUCUCCAAAGGAUCUCGAAUU	594	stab22	AAA1-4:691L21 antisense siNA (673C)
873	AAUGACACCCUUGGAUCUGAACCA	595	AAA1-4:893L21 antisense siNA (875C)	GCUGAAAGGAUGCAUAGCAGTT B
897	UACACACCGGACAAUUGUGUGC	596	stab22	AAA1-4:917L21 antisense siNA (899C)
				ACACAAUUGGUCCUGGUGGTT B
				788

Uppercase = ribonucleotide  
 u,c = 2'-deoxy-2'-fluoro U,C

T = thymidine

B = inverted deoxy abasic  
 s = phosphorothioate linkage

A = deoxy Adenosine

G = deoxy Guanosine

G = 2'-O-methyl Guanosine  
A = 2'-O-methyl Adenosine

Table IV

Non-limiting examples of Stabilization Chemistries for chemically modified siNA constructs

Chemistry	pyrimidine	Purine	Cap	p=S	Strand
“Stab 00”	Ribo	Ribo	TT at 3'-ends		S/AS
“Stab 1”	Ribo	Ribo	-	5 at 5'-end 1 at 3'-end	S/AS
“Stab 2”	Ribo	Ribo	-	All linkages	Usually AS
“Stab 3”	2'-fluoro	Ribo	-	4 at 5'-end 4 at 3'-end	Usually S
“Stab 4”	2'-fluoro	Ribo	5' and 3'-ends	-	Usually S
“Stab 5”	2'-fluoro	Ribo	-	1 at 3'-end	Usually AS
“Stab 6”	2'-O-Methyl	Ribo	5' and 3'-ends	-	Usually S
“Stab 7”	2'-fluoro	2'-deoxy	5' and 3'-ends	-	Usually S
“Stab 8”	2'-fluoro	2'-O-Methyl	-	1 at 3'-end	S/AS
“Stab 9”	Ribo	Ribo	5' and 3'-ends	-	Usually S
“Stab 10”	Ribo	Ribo	-	1 at 3'-end	Usually AS
“Stab 11”	2'-fluoro	2'-deoxy	-	1 at 3'-end	Usually AS
“Stab 12”	2'-fluoro	LNA	5' and 3'-ends		Usually S
“Stab 13”	2'-fluoro	LNA		1 at 3'-end	Usually AS
“Stab 14”	2'-fluoro	2'-deoxy		2 at 5'-end 1 at 3'-end	Usually AS
“Stab 15”	2'-deoxy	2'-deoxy		2 at 5'-end 1 at 3'-end	Usually AS
“Stab 16”	Ribo	2'-O-Methyl	5' and 3'-ends		Usually S
“Stab 17”	2'-O-Methyl	2'-O-Methyl	5' and 3'-ends		Usually S
“Stab 18”	2'-fluoro	2'-O-Methyl	5' and 3'-ends		Usually S
“Stab 19”	2'-fluoro	2'-O-Methyl	3'-end		S/AS
“Stab 20”	2'-fluoro	2'-deoxy	3'-end		Usually AS
“Stab 21”	2'-fluoro	Ribo	3'-end		Usually AS
“Stab 22”	Ribo	Ribo	3'-end		Usually AS
“Stab 23”	2'-fluoro*	2'-deoxy*	5' and 3'-ends		Usually S
“Stab 24”	2'-fluoro*	2'-O-Methyl*	-	1 at 3'-end	S/AS
“Stab 25”	2'-fluoro*	2'-O-Methyl*	-	1 at 3'-end	S/AS

“ <b>Stab 26</b> ”	2'-fluoro*	2'-O-Methyl*	-		S/AS
“ <b>Stab 27</b> ”	2'-fluoro*	2'-O-Methyl*	3'-end		S/AS
“ <b>Stab 28</b> ”	2'-fluoro*	2'-O-Methyl*	3'-end		S/AS
“ <b>Stab 29</b> ”	2'-fluoro*	2'-O-Methyl*		1 at 3'-end	S/AS
“ <b>Stab 30</b> ”	2'-fluoro*	2'-O-Methyl*			S/AS
“ <b>Stab 31</b> ”	2'-fluoro*	2'-O-Methyl*	3'-end		S/AS
“ <b>Stab 32</b> ”	2'-fluoro	2'-O-Methyl			S/AS

CAP = any terminal cap, see for example **Figure 10**.

All Stab 00-32 chemistries can comprise 3'-terminal thymidine (TT) residues

All Stab 00-32 chemistries typically comprise about 21 nucleotides, but can vary as described herein.

5

S = sense strand

AS = antisense strand

\*Stab 23 has a single ribonucleotide adjacent to 3'-CAP

\*Stab 24 and Stab 28 have a single ribonucleotide at 5'-terminus

10

\*Stab 25, Stab 26, and Stab 27 have three ribonucleotides at 5'-terminus

\*Stab 29, Stab 30, and Stab 31, any purine at first three nucleotide positions from 5'-terminus are ribonucleotides

p = phosphorothioate linkage

15

Table V

A. 2.5  $\mu$ mol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	6.5	163 $\mu$ L	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 $\mu$ L	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 $\mu$ L	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 $\mu$ L	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
Iodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucage	12.9	645 $\mu$ L	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA	NA	NA

B. 0.2  $\mu$ mol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	15	31 $\mu$ L	45 sec	233 sec	465 sec
S-Ethyl Tetrazole	38.7	31 $\mu$ L	45 sec	233 min	465 sec
Acetic Anhydride	655	124 $\mu$ L	5 sec	5 sec	5 sec
N-Methyl Imidazole	1245	124 $\mu$ L	5 sec	5 sec	5 sec
TCA	700	732 $\mu$ L	10 sec	10 sec	10 sec
Iodine	20.6	244 $\mu$ L	15 sec	15 sec	15 sec
Beaucage	7.7	232 $\mu$ L	100 sec	300 sec	300 sec
Acetonitrile	NA	2.64 mL	NA	NA	NA

C. 0.2  $\mu$ mol Synthesis Cycle 96 well Instrument

Reagent	Equivalents:DNA/ 2'-O-methyl/Ribo	Amount: DNA/2'-O- methyl/Ribo	Wait Time* DNA	Wait Time* 2'-O- methyl	Wait Time* Ribo
Phosphoramidites	22/33/66	40/60/120 $\mu$ L	60 sec	180 sec	360sec
S-Ethyl Tetrazole	70/105/210	40/60/120 $\mu$ L	60 sec	180 min	360 sec
Acetic Anhydride	265/265/265	50/50/50 $\mu$ L	10 sec	10 sec	10 sec
N-Methyl Imidazole	502/502/502	50/50/50 $\mu$ L	10 sec	10 sec	10 sec
TCA	238/475/475	250/500/500 $\mu$ L	15 sec	15 sec	15 sec
Iodine	6.8/6.8/6.8	80/80/80 $\mu$ L	30 sec	30 sec	30 sec
Beaucage	34/51/51	80/120/120	100 sec	200 sec	200 sec
Acetonitrile	NA	1150/1150/1150 $\mu$ L	NA	NA	NA

5 • Wait time does not include contact time during delivery.

• Tandem synthesis utilizes double coupling of linker molecule